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THE REACTION OF NORMOBLASTIC BONE MARROW TO LIVER EXTRACT*

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RECENTLY there appeared an article by Jacobson[1] describing the reticulocyte response of normal guinea pigs to the oral and parenteral administration of liver extracts. His experiments were performed in an attempt to find a suitable method for assaying the potencies of the various therapeutic substances used in the treatment of macrocytic hyperchromic anemias. The work is similar to that of Vaughan, Muller, and Zetzel[2] and Vaughan, Muller and Minot[3] with pigeons. It is maintained that the pigeon and the guinea pig respond to various liver extracts because they normally possess a megaloblastic bone marrow comparable to that found in the human being with pernicious anemia during relapse, and that it is abnormal for the pigeon to possess a normoblastic bone marrow.[1, 4-6] It is claimed further that the failure of a reticulocyte response in rats to substances effective in treating pernicious anemia is due to the absence of a megaloblastic bone marrow.[7] In a recent monograph Singer[8] has shown conclusively that not only does the normal rat respond to injections of liver extract but also to injections of unconcentrated human gastric juice. Since rats will not elicit a reticulocyte response to gastric juice from pernicious anemia patients, Singer maintains that this method can be used as a biologic test for pernicious anemia. Recently Wills and others[9, 10] have discredited the above conclusion regarding the reticulocyte

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response in normal pigeons to liver extract as a method of assay, by showing that the response is within the normal range of reticulocyte percentage for the pigeon.

If it were true that the normal marrow of these animals is megaloblastic, much could be learned concerning the origin of the megaloblast, its relation to the definitive red blood corpuscle, and the reason for its disappearance after liver therapy.

With such thoughts in mind the author duplicated some of the experiments of Jacobson[1] and in addition examined the bone marrow of guinea pigs at various stages of erythropoietic activity following the parenteral administration of liver extract.*

A series of bone marrows from a control group of guinea pigs was examined in a similar manner. In addition, marrow from one- and three-day-old pigeons, from adult pigeons, and from adult rats was examined. The preparations were of the "abklatsch" or imprint type, and they were stained with the May-Grünwald-Giemsa combination of Pappenheim. This work has been at all times correlated with studies of human pernicious anemia bone marrow.

Examination was made of all the bone marrows for the earliest developmental stages of the red cell series. In the guinea pig, pigeon, and rat these were found to be the pronormoblasts (proerythroblasts) which are identical with similar cell types found in normal human marrow. This fits in with the findings of Minot[11] who described the normoblastic series of the mammalian red blood corpuscles as being sauroid, meaning that the mammalian definitive series originated from cells which were similar to those found in the circulating blood of the birds and reptiles. This cell type has been adequately described and pictured by Ferrata,[12] Naegeli,[13] and Negreiros-Rinaldi.[14]

In the biopsied pernicious anemia bone marrow the earliest red cells are predominantly of the megaloblastic type rather than of the normoblastic type. That is to say, that the promegaloblasts greatly outnumber the pronormoblasts. The differences between these cell types have been described by the authors mentioned above[12-14] and by others. A thorough examination was made for a cell possessing megaloblastic characteristics while studying the guinea pig, pigeon, and rat bone marrow. No cell belonging to the megaloblastic series could be found in any of the marrows examined. The red cells of these marrows, therefore, belong to the definitive or normoblastic series, and they are identical with those of the marrow of the normal human adult which does not possess megaloblasts.[15]

Considerable confusion in this problem has been due to misusage of the terminology and to faulty technic. Doan, Cunningham and Sabin[16] redefined the term megaloblast to mean something entirely different from its original application. Ehrlich and Lazarus[17] stated that "when a name has been suggested for a definite matter, this name should not be employed to signify some other phenomenon or structure. This claim has not been respected in modern hematology. When an investigator considers that the suggested term is un-

*Lederle's Solution Liver Extract (Parenteral).

suitable in any respect, he can propose a new, better term, but he should not create confusion by the false use of the original name."

Some of the confusion concerning the megaloblast-normoblast problem is due to the various technics used.[12-15, 18-21] From our own experience it is felt that the methods used by Doan and Zerfas[22] and Isaacs,[23] which depend on the emulsification of the marrow, are adequate for use in an attempt to make a differential count of marrow cells. However, we do not believe that they bring out the finer cytologic detail of the nucleus as well as the dry "abklatsch" or imprint preparations stained with May-Grünwald-Giemsa. The use of sections only (Turnbull[21]) has the disadvantage of not demonstrating the delicate nuclear structures in the very young cells, as pointed out by Downey.[24] The inadequacy of supravital staining as used by Doan, Cunningham and Sabin[16] and Doan[20] in studying the finer morphologic details is discussed by Hall.[25] We find that the imprint or "abklatsch" method used by Pappenheim, Ferrata, Naegeli, and Downey is the best suited for studying the cellular relationships of bone marrow components.

Our experiments show there is a significant reticulocyte response by the guinea pig to parenteral liver extract as described by Jacobson,[1] but that this response cannot be attributed to a megaloblastic bone marrow of the guinea pig, since we have shown that this marrow is normoblastic rather than megaloblastic. In human beings those possessing a pathologic bone marrow, of the type found in pernicious anemia, respond to liver extract, while, on the other hand, in guinea pigs the normoblastic (proerythroblastic) bone marrow responds. The response to the same antipernicious anemia-principle in different species of animals not having a megaloblastic marrow emphasizes the fact that generalizations concerning the reticulocyte response to liver extract in pernicious anemia cannot be carried over to the other mammals without obtaining substantial morphologic evidence for the similarity of the corresponding hematopoietic tissue. Since it cannot be shown that the test animals possess a megaloblastic bone marrow identical with that of pernicious anemia patients, we must conclude that the response is not due to a similarity of the bone marrows, but rather to an inexplicable species difference morphologically unexplained at present.

We find no morphologic evidence for a megaloblastic bone marrow in the guinea pig. The earliest forms of the red cell series in this animal are identical with those found in the normal human adult. In spite of the fact that the reticulocyte response in the normal guinea pig to oral or parenteral administration of liver extract is conditioned by a totally different type of bone marrow than in human beings with pernicious anemia, this method of assay of the therapeutic potencies of various substances may still prove to be of great practical value.

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ADDENDA

Just recently Jacobson has published an article which reiterates his speculations concerning the cause for the reticulocyte response in guinea pigs to substances effective in treating pernicious anemia (J. Clin. Investigation 14: 664, 1935). He maintains that "The large number of megaloblasts, in proportion to the number of normoblasts, simulates the classical picture of the bone marrow findings in pernicious anemia." In other words, the guinea pig normally possesses a bone marrow which is encountered only under pathologic conditions in the human being.

In order to illustrate the differences between these two bone marrows and to show that they are not identical nor simulate one another, reference should be made to Figs. 1 and 2. The center cell in Fig. 1 is a promegaloblast, while the

two adjacent cells are basophilic megaloblasts. These cells are in no way related to the definitive red blood cell series nor are they found in normal marrow, whether it be guinea pig or human being.[15] The field in Fig. 2 represents two cells belonging to the first developmental stage of the red blood cell series found in normal guinea pig bone marrow. These cells are pronormoblasts (proerythro-

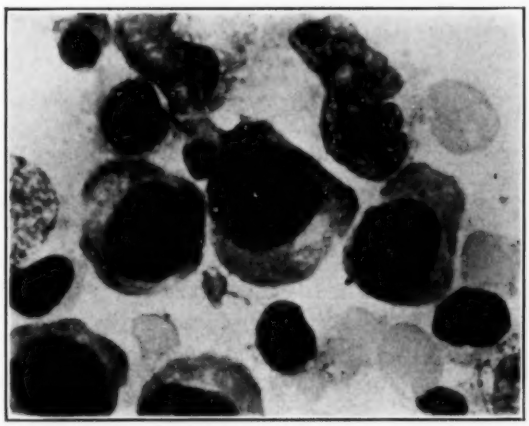

Fig. 1.—The center cell is a promegaloblast, while the two adjacent cells are basophilic megaloblasts. This is dry imprint preparation of biopsied pernicious anemia bone marrow during relapse. May-Grünwald-Giemsa stain. Magnification ×1500.

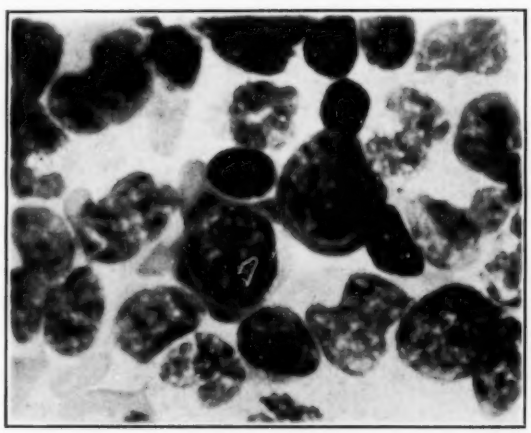

Fig. 2.—The cells in the center of the field are pronormoblasts (proerythroblasts) from a dry imprint preparation of normal guinea pig bone marrow. Nucleoli are present in both cells. Stain and magnification are the same as in Fig. 1.

blasts) and have been misinterpreted as "megaloblasts" by many authors. Note the difference in arrangement of chromatin as well as the nucleoplasma ratio (in the megaloblasts and normoblasts). The cells found in Fig. 2 are identical to the first developmental stage of the red blood cell series encountered in normal human bone marrow.

STUDIES OF PLASMA PROTEINS AND CHOLESTEROL*

In Normal White and Colored Individuals, and in Negroes with Arteriosclerosis

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INTRODUCTION

WHEN the medical experience of one of us began to include more clinical examinations of negroes some two or more years ago, it was apparent that arteriosclerosis was encountered both more frequently and at a much earlier age than in white patients. A study, recently completed, was made of the incidence of this finding in patients under the age of forty who presented no evidence of hypertension nor nephritis. Naturally, the question of etiology of vascular changes in young negroes arose. Since inadequacy of nutrition seemed possible in the face of the circumscribed diet of so many negroes, we began a study of the cholesterol and proteins of the blood plasma.

Fortunately, two of us had already made a study of these plasma components in a group of thirty-five healthy medical students. This group offered a satisfactory control for the studies on thirty-one negroes, one-half of whom presented arteriosclerosis, while the other half were apparently well-nourished young negroes complaining of minor disturbances.

EXPERIMENTAL

All determinations were made on oxalated plasma, using the minimum amount of dry potassium oxalate necessary to prevent clotting. All analyses were started within a few hours after the blood samples were taken; in a few instances, duplicate determinations were carried out. If there seemed to be any reason for the determination being inaccurate, it was either discarded or repeated.

The plasma proteins were determined after the method of Wu[1] as modified by Andersch and Gibson.[2] This modification seemed to be very satisfactory in our hands, and where duplicate determinations were made, the agreement was all that could be desired. The plasma cholesterol was determined by the method of Myers and Wardell[3] as modified by Andes.[4] In most cases whole blood cholesterol was also estimated as a check. In Table I are given the results of plasma proteins and cholesterol in thirty-five healthy white persons. All but two were either medical students or technicians, between the ages of eighteen and twenty-nine years. Twenty-six were males. Two white females, aged forty-one and

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fifty years, were included in this group. Practically all of this group had been examined physically by members of the Department of Medicine at the beginning of the school year. Urine examination and blood pressure determinations in all were negative. The results of this study are summarized at the end of Table I.

The results of plasma protein and cholesterol determination in sixteen negro males showing arteriosclerosis are presented in Table II. All of the patients were ambulant and attended the Medical Out-Patient Department of Charity Hospital. Vascular disease was judged on the degree of sclerosis, incompressibility and tortuosity of the radial and brachial arteries and in some, of the dorsalis pedis artery. Visible pulsation of the radial arteries appeared rather frequently. The ages of the group ranged as follows: one was sixteen years of age, eleven fell between the ages of twenty to thirty years, and four were between the thirtieth and fortieth years.

TABLE I

BLOOD CHOLESTEROL AND PLASMA PROTEINS OF NORMAL INDIVIDUALS

NO.	AGE AND SEX	PLASMA CHOLESTEROL	PLASMA PROTEINS				
			TOTAL	FIBRINOGEN	ALBUMIN	GLOBULIN (SERUM)	A/G RATIO*
1	20 M	159	7.63	0.27	4.83	2.53	1.73
2	18 F	193	8.19	0.33	5.37	2.50	1.90
3	25 M	193	7.13	0.39	4.09	2.83	1.27
4	26 M	188	8.59	0.52	5.01	3.06	1.37
5	20 M	179	8.42	0.27	5.56	2.59	1.94
6	25 M	170	7.69	0.31	4.49	2.69	1.50
7	22 M	172	8.51	0.32	5.73	2.56	2.00
8	18 M	140	8.39	0.29	5.06	3.04	1.52
9	23 M	185	7.25	0.30	4.45	2.50	1.59
10	22 M	162	8.59	0.31	5.52	2.76	1.80
11	25 M	142	8.93	0.38	5.27	3.28	1.44
12	24 M	225[3]	8.15	0.23	4.96	2.96	1.55
13	20 M	141	8.04	0.21	4.88	2.95	1.54
14	26 M	174	8.77	0.37	5.60	2.80	1.77
15	19 F	188	7.73	0.32	5.06	2.35	1.90
16	24 F	167	7.67[1]		5.04	2.63	1.72[2]
17	24 M	175	7.67[1]		5.16	2.63	1.76[2]
18	22 M	151	7.25[1]		4.60	2.65	1.56[2]
19	29 M	173	7.87[1]		4.96	2.91	1.55[2]
20	27 M	140	7.69	0.25	4.53	2.91	1.43
21	26 F	175	6.97	0.36	3.85	2.56	1.32
22	18 M	145	8.01	0.35	4.78	2.88	1.48
23	22 M	160	7.40	0.24	4.68	2.48	1.72
24	20 F	160	8.77	0.28	5.16	3.33	1.43
25	23 M	148	8.47	0.27	5.37	2.83	1.73
26	24 M	158	7.27	0.27	4.37	2.63	1.51
27	23 M	144	8.79	0.33	4.96	3.50	1.30
28	25 F	151	9.15	0.31	5.16	3.68	1.30
29	24 M	193	7.79	0.25	4.78	2.76	1.59
30	26 M	146	8.17	0.34	5.30	2.53	1.85
31	22 M	199	7.82	0.37	4.69	2.76	1.50
32	25 F	150	8.83	0.37	5.27	3.56	1.34
33	41 F	203	7.62	0.48	4.45	2.69	1.40
34	23 F	150	8.63[1]		5.17	3.46	1.38[2]
35	50 F	207	8.27	0.20	5.40	2.67	1.88
High	50	207 (225)	9.15	0.52	5.60	3.68	2.00
Low	18	140	6.97	0.20	3.85	2.35	1.27
Av.	24	167	8.06	0.32	4.94	2.85	1.56

*Where G represents serum globulin and fibrinogen (plasma globulin).

[1]Value is for total serum proteins.

[2]Calculated assuming the fibrinogen to be 0.30 gm.

[3]This figure is excluded in the final average

From the diagnoses listed in the table, it is evident that, generally speaking, the determinations were made in patients without any serious infection known to cause deviation of plasma proteins from normal. However, two cases of minimal or moderately advanced pulmonary tuberculosis, and three cases of treated syphilis were included. But from the table it may be seen that these

TABLE II

PLASMA CHOLESTEROL AND PROTEINS IN NEGRO MALES WITH ARTERIOSCLEROSIS

NO.	AGE	DIAGNOSIS	PLASMA CHOLESTEROL	PLASMA PROTEINS				
				TOTAL	FIBRINOGEN	ALBUMIN	GLOBULIN (SERUM)	A/G RATIO*
1	29	Sacroiliac strain		8.16	0.43	4.69	3.04	1.35
2	30	No disease	132	8.74	0.40	5.16	3.18	1.44
3	29	Pulmonary tuberculosis	192	8.37	0.60	3.95	3.82	0.89
4	24	Latent syphilis		8.23	0.46	3.97	3.80	0.93
5	33	Pharyngitis	145	8.40	0.34	4.10	3.96	0.95
6	38	Generalized arteriosclerosis	169	7.50	0.35	3.97	3.28	1.09
7	28	Constipation	173	7.83	0.36	3.97	3.50	1.03
8	27	No disease		7.79	0.30	4.10	3.39	1.11
9	29	Hyperchlorhydria	210	9.89	0.61	4.62	4.66	0.88
10	16	Idiopathic epilepsy		7.82	0.37	4.45	3.00	1.32
11	35	Headache	163	6.52	0.33	3.04	3.15	0.87
12	26	Pleurisy	130	8.39	0.67	3.69	4.04	0.78
13	29	Syphilis (treated)		9.11	0.49	5.49	3.13	1.52
14	38	Aortic insufficiency	150	8.56	0.64	4.30	3.62	1.01
15	28	Pulmonary tuberculosis	157	8.06	0.54	4.23	3.29	1.10
16	30	No disease	162	8.83	0.57	4.23	4.04	0.92
High	38		210	9.11	0.67	5.49	4.66	1.52
Low	16		132	6.52	0.30	3.04	3.00	0.78
Av.	29		162	8.26	0.47	4.25	3.54	1.07

*Where G represents serum globulin + fibrinogen (plasma globulin).

TABLE III

PLASMA CHOLESTEROL AND PROTEINS OF NORMAL NEGRO MALES

NO.	AGE	DIAGNOSIS	PLASMA CHOLESTEROL	PLASMA PROTEINS				
				TOTAL	FIBRINOGEN	ALBUMIN	GLOBULIN (SERUM)	A/G RATIO*
1	28	Myalgia	148	7.71	0.20	4.23	3.28	1.21
2	28	Headache	140	8.11	0.40	4.16	3.55	1.05
3	14	No disease	155	7.37	0.39	4.03	2.95	1.21
4	11	Rhinitis	146	7.27	0.36	3.91	3.00	1.16
5	9	No disease	160	8.18	0.51	4.23	3.44	1.07
6	23	Hyperchlorhydria	158	7.75	0.32	4.30	3.13	1.24
7	17	Intercostal neuralgia	167	8.32	0.37	3.91	4.04	0.88
8	19	Radiculitis	154	7.30	0.30	4.30	2.70	1.43
9	24	Sacroiliac strain	164	9.99	0.59	4.74	4.66	0.93
10	19	Acute bronchitis	171	8.20	0.40	4.30	3.50	1.10
11	27	Sacroiliac strain	180	8.15	0.37	4.78	3.00	1.42
12	15	Tracheobronchial lymphadenopathy	165	8.81	0.54	5.27	3.00	1.49
13	23	Myalgia	178	7.52	0.36	4.16	3.00	1.24
14	14	Acute bronchitis	136	10.52	0.86	4.78	4.88	0.83
15	31	No disease	167	9.09	0.56	4.78	3.75	1.11
High	31		180	10.52	0.96	5.27	4.88	1.59
Low	9		132	7.27	0.20	3.49	2.70	0.83
Av.	20		155	8.32	0.47	4.34	3.52	1.16

*Where G represents total globulin (fibrinogen + serum globulin).

patients by no means show the greatest deviation from normal and therefore do not vitiate the results. It must be emphasized that patients were chosen in whom there was no gross evidence of undernutrition and in whom there was no history or physical evidence of nutritional edema.

The morning urine was negative in every case, in none was the specific gravity below 1.015, the majority having a gravity of over 1.020. Albuminuria and cylindruria were absent. Blood pressure generally ranged from systolic 105 to 135 mm. and diastolic 70 to 90 mm. of mercury. The highest systolic pressure was 145 mm. in a case of aortic insufficiency. Average pressures for the sixteen cases were systolic 123 mm. and diastolic 75 mm. of mercury. The Wassermann reaction was positive in two cases and negative in the rest of the group.

The deviation of plasma proteins in the arteriosclerosis group from the normal values of the white medical students, obviously, demanded similar studies in a group of young negroes without vascular disease, since we did not seriously relate arteriosclerosis to abnormal protein findings. Table III shows the results of the determination in fifteen young ambulant negro males, who presented no evidence of vascular disease.

Generally, these were younger than in the other group, the ages being as follows: one was nine years of age, seven were between ten and twenty years of age, six fell between the twentieth and thirtieth years, and one was thirty-one years old. From the diagnoses in Table III, it is obvious that none of the patients suffered from serious disease. A history of syphilis was absent in every case. In this group, especially, patients were chosen who appeared well-nourished, and gave no history nor showed any findings of any type of edema.

The urine was negative in all respects in every case, a satisfactory specific gravity for a single specimen being present in all. The highest blood pressure recorded was systolic 135 mm. and diastolic 85 mm. of mercury. Average of all pressure readings was systolic 113 mm. and diastolic 75 mm. of mercury. Blood Wassermann reaction was negative.

DISCUSSION

The results of the study of the normal white individuals can be briefly considered. The cholesterol range of 140 to 207 mg./100 c.c., with an average of 167 mg. agrees with the commonly accepted range very closely. The values for the total proteins (approximately 7 to 9 gm./100 c.c.) compare very well with the accepted normal range, although being slightly higher. The albumin and globulin values need no comment. The A/G ratio (1.3-2.0) agrees very favorably with the ratios given by Linder,[5] Salversen,[6] and other recent workers. The fibrinogen values practically all fall within the accepted range of 0.2 to 0.4 gm./100 c.c.

In the negro patients (Tables II and III), both the normal ones and those with arteriosclerosis, the plasma cholesterol can be regarded as entirely normal. The slightly lower average figures, as well as slightly lower range, are not enough to be significant. These normal figures agree with the results of Bloor,[7] Dennis,[8] and the majority of other workers.

With the plasma proteins, however, an entirely different state of affairs is found. Although the total protein is little different from that of the normal white group, the individual constituents vary considerably (a slight increase is

observed in the average values in both groups). The fibrinogen and serum globulin values are definitely increased, while the albumin is lowered. This produces a fairly marked reduction in the A/G (albumin/globulin) ratio. In fact, the ratios are similar to those found in mild nephritic and nutritional edema (see Table IV), though no history nor evidence of edema was present. Furthermore no marked difference is present between the group of normal negroes and those with arteriosclerosis. The total proteins and fibrinogen values are almost identical, while the A/G ratio is slightly lower in the arteriosclerosis group.

Explanation of the findings in the negroes is at this time impossible. Patients were selected who were apparently well nourished. It is evident to everyone that the diet of the average Southern negro is not as well balanced as the accepted dietary demand. At first an attempt was made to gain information about the diet from each patient. This was soon abandoned, however, because of the low mental state and lack of cooperation of so many of the patients. Therefore, an idea of the average diet of the negro was obtained by questioning the more cooperative patients, from the information given by a rural storekeeper, and from Dr. P. B. Cameron, who conducts the Diabetic Clinic at Charity Hospital. These sources all agree on the constituents of the negro diet. Fresh meat is available only once or twice a week, at the most, and then only in small quantities. Eggs are used to some extent; also milk, though the latter, in the urban districts, may be of poor quality. Carbohydrate is high in the diet, in the form of grits, cornbread, white bread, rice, and red and navy beans. The leafy vegetables are represented mainly by collard, mustard or turnip greens. Caloric intake and the distribution of the above constituents of the diet no doubt vary in the individual cases on the basis of economic considerations.

COMMENT

The results of our studies are of especial interest since they may indicate a nutritional lack before it is manifest clinically, a "nutritional pre-edema state." We feel that these findings should be called to the attention of students interested in serum protein studies. Our results show that nutritional deficiency may make itself manifest by a change in the serum proteins, although not of such degree as to lower the serum osmotic pressure sufficiently to produce edema.

In order to compare our findings with those found in nutritional edema, we have arranged our average figures together with those of several other investigators (Table IV).

It is apparent that the total protein concentration in our negro groups is higher than in the normal, as a result of the increase in the globulin fraction. Since the decrease in the albumin is not very marked, the globulin increase has maintained the osmotic pressure high enough so that edema cannot occur. In fact, if the fibrinogen is added to the calculations of osmotic pressure, the pressures of the normal white and the negro groups are almost identical (40.7 and 39.1 cm. of water, respectively). It would seem, therefore, that in our patients the fall in albumin has started as a result of certain dietary deficiencies, but that a compensatory globulin increase has occurred which keeps the osmotic pressure approximately normal.

This is in contrast with most of the other values reported in malnutrition (as shown in Table IV), although all cases with edema show some lowering in the A/G ratio, as a result of a slower fall in the globulin fraction. It would seem that our cases fall in the region between the normal and those persons with frank protein shortage.

In explaining nutritional edema, others have discussed the matter of total nitrogen intake. We should like to view the question from a slightly different angle.

TABLE IV

AVERAGE SERUM PROTEIN VALUES

TYPE OF CASE	AUTHORS	NO. OF CASES	SERUM PROTEINS			A/G RATIO*	OSMOTIC PRESSURE IN CM. H_2O†
			TOTAL	ALBUMIN	GLOBULIN		
Normal white individuals	Andes, Kampmeier, and Adams	35	7.74	4.94	2.85	1.73	39.1
Normal negroes	Andes, Kampmeier, and Adams	15	7.85	4.34	3.52	1.23	36.9
Negroes with arteriosclerosis	Andes, Kampmeier, and Adams	16	7.79	4.25	3.54	1.20	36.2
Nutritional edema	Youmans and associates[9]	31	6.95	3.98	2.89	1.38	31.2
Malnutrition without edema	Bruckman and Peters[10]	41	6.17	3.82	2.43	1.57	27.1
Malnutrition with edema	Bruckman and Peters[10]	18	5.75	3.16	2.64	1.20	23.0
Malnutrition without edema	Bruckman, D'Esopo and Peters[11]	42‡	6.20	3.86	2.41	1.60	27.4
Malnutrition with edema	Bruckman, D'Esopo and Peters[11]	18‡	5.50	3.16	2.69	1.17	22.0

*Where G = serum globulin.
†Calculated according to the formula of Wells, Youmans and Miller.[12]
‡A larger number of cases were analyzed for the total proteins.

Since bread and beans make up such a large proportion of the diet of the negro, the total quantity of protein consumed is probably adequate in all cases. However, the biologic value of these proteins is not very high [52 per cent for white bread and 38 per cent for beans[13]]. Also a consideration of the amino acid content of the proteins of white bread and brown beans reveals the fact that white bread proteins are deficient in cystine and lysine, and bean proteins are deficient in cystine. In view of the fact that serum globulin contains only about one-half as much cystine and two-thirds as much lysine as does albumin,[14] it seems plausible to believe that the body can more easily synthesize globulin than albumin, when the food source is deficient in these two amino acids. This would explain why the plasma globulin is increased, even though the albumin has decreased.

Since the osmotic pressure of globulin is much lower than albumin, any osmotic deficiency due to lack of albumin is made up only by an increase in globulin several times the magnitude of the albumin deficit. This would account for the high total proteins.

In addition, the diet is unquestionably low in vitamins A, C, and D, and the absence of these accessory food factors may be a factor in the genesis of the abnormal protein concentration.

The small differences between the plasma protein figures in normal negroes and those with arteriosclerosis are not great enough to be very significant, although a difference exists. Furthermore, any relation between plasma proteins and arteriosclerosis cannot be regarded as established, although the figures presented do not in any sense rule out such a possibility.

SUMMARY

1. Values for the plasma protein constituents and plasma cholesterol are given for 35 normal white persons, 16 young negroes with arteriosclerosis and 16 young negroes without evidence of vascular disease.

2. Both the arteriosclerosis and normal negro groups showed findings for the above constituents so similar as to be considered as a single group, the arteriosclerosis group showing the greater deviation from the normal.

3. The principal changes in the negro patients were (a) slightly increased total proteins; (b) definitely increased fibrinogen and serum globulin; and (c) lowered albumin concentration.

4. The A/G ratio was therefore markedly decreased, being similar to, though not as marked as, that commonly observed in nephritic or nutritional edema. However, nephritis and nutritional edema were not present in our material.

5. The results of this study suggest a "nutritional pre-edema" stage.

6. The abnormal protein findings are explained on the basis of a qualitative protein deficiency, although the explanation is merely a hypothesis on our part.

7. Cholesterol values were essentially the same in all three groups, no significant alteration being found in the group with arteriosclerosis.

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THE RELATION OF THE THYROID GLAND TO HEMATOPOIESIS*

I. Experimental Total Thyroidectomy in the Rabbit

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WE BECAME interested in the relationship of the thyroid gland to hematopoiesis for the following reasons: (1) The close clinical similarity between chronic lymphatic leucemia and the exophthalmic type of hyperthyroidism. (2) The experimental work of Gottlieb[1] who showed that the number of lymphocytes in the blood of the thyroid vein was 30 per cent higher than that in the blood of the thyroid artery or the systemic capillary system. (3) The report of a case of lymphatic leucemia in which a total ablation of the thyroid gland was performed with startling disappearance of the signs of the disease. (4) The development of anemia in spontaneous and postoperative myxedema.

Thyroid Gland and Leucemia.—Clinically, chronic lymphatic leucemia and hyperthyroidism are very much alike. Symptoms which are characteristic of both diseases include fatigue, loss of weight, tachycardia, perspiration, and tolerance to cold; they are, of course, more prominent in hyperthyroidism. As in exophthalmic goiter, leucemic patients may also present such symptoms as an increased appetite, flushing of the skin, diarrhea, menstrual disturbances, nervousness, irritability, and insomnia. Bilateral exophthalmos has even been reported by Friedgood[2] in five of his ten cases of lymphatic leucemia. Minot and Means[3] had previously called attention to the presence of unilateral exophthalmos in leucemia, and in addition, to a fine tremor of the hands which they attribute to the associated anemia. Generalized lymphadenopathy and splenomegaly, so characteristic of lymphatic leucemia, are present, but to a lesser degree, in 30 per cent of the cases of hyperthyroidism.[4-7] The etiology of the enlargement of the lymph nodes and spleen is not known, but it is probably a part of the generalized lymphoid hyperplasia which also gives rise to the characteristic and well-known pathologic picture of lymphoid hyperplasia seen in the thyroid gland of exophthalmic goiter. Further evidence of this lymphoid activity is reflected in the study of the blood. The Kocher[8] blood picture, viz., leucopenia and relative lymphocytosis, is still accepted by most clinicians.[1, 6, 7, 9-11] Menkin[11] again recently stressed the finding of relative lymphocytosis in 67 per cent of the cases of hyperthyroidism that he studied. There is a difference of opinion as to what effect iodine has on the blood picture of hyperthyroidism. Jackson[12] states that it has no effect; Hertz and Lerman[9] that it has a definite depressing influence on the mononuclear elements. It is significant, however, to note that all agree that thyroidectomy restores the normal differential formula of the blood.[9, 11, 12] Caro[13]

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described a case of fatal exophthalmic goiter with such atypical findings in the blood that he thought he was dealing with an associated pseudoleucemia. He later collected several other cases showing similar blood pictures. It is not necessary to discuss the characteristic blood picture of lymphatic leucemia, but we think it pertinent to point out a rough similarity in the relative lymphocytosis of hyperthyroidism and of the aleucemic phase of lymphatic leucemia. The basal metabolic rate is increased not only in hyperthyroidism, but also in cases of both lymphatic and myelogenous leucemia.[3, 14, 15] In fact, Friedgood[16] has observed an increase in the basal metabolic rate in certain cases of acromegaly, diabetes mellitus, essential hypertension, polycythemia vera, and pernicious anemia. Just as the cause of the increased oxygen consumption in hyperthyroidism is, for the most part, speculative, so is the explanation of the increased metabolic rate in leucemia. It has been shown that the height of the basal metabolic rate in leucemia is not necessarily related to the height of the leucocyte count, but more closely comparable to the patient's general condition, the pulse rate, the degree of anemia and inversely to the number of platelets of the blood.[14, 15] Some authorities believe that in leucemia the level of the basal metabolic rate is of more prognostic value than the number of the leucocytes.[15] The well-defined and favorable effect of Lugol's solution in exophthalmic goiter is also seen, but to a lesser degree, in leucemia. Friedgood[17] has noted a definite decrease in the nervousness and in the fatigue of leucemic patients following the administration of Lugol's solution. Such favorable effects of iodine as reductions of the basal metabolic rate, the pulse rate, the lymphocytes of the blood and the size of the enlarged lymph nodes and spleen are variable. In a certain percentage of patients, there was a definite symptomatic remission, but in others there was no effect. The action of Lugol's does not seem to be due to either the reduction of the basal metabolic rate, or any influence exerted by the thyroid gland, but through a "sedative" effect on a hyperactive state of the sympathetic nervous system. So, in summing up a comparison of the two diseases, chronic lymphatic leucemia and exophthalmic goiter, we find not only many similar signs and symptoms, but, in addition, that both have generalized lymphoid hyperplasia, that both have an increase in circulatory lymphocytes, and that both have an increased basal metabolic rate which responds beneficially to Lugol's solution. The main difference in these factors in the two diseases, as pointed out by Friedgood,[17] is not qualitative but rather quantitative.

Thyroidectomy and Leucemia.—The suggestion of total thyroidectomy for the treatment of lymphatic leucemia may appear, at first glance, to be the result of a wave of impulsive, enthusiastic surgery that usually follows any new procedure such as has recently been advanced by Blumgart and his associates[18] in the treatment of angina pectoris and congestive heart failure. However, from the foregoing discussion there is evidence to indicate that removal of the thyroid gland in lymphatic leucemia might have a beneficial effect, certainly enough to warrant its therapeutic trial in this otherwise fatal disease. Dameshek and coworkers[19] reported such a case in a forty-two-year-old woman with all the signs of an aleucemic lymphatic leucemia with a basal metabolic rate of plus 65. It was thought that the continued loss of weight, profuse drenching sweats, increasing nervous

symptoms, and the beginning circulatory failure might be due to the markedly increased metabolic rate. When the patient became extremely ill and failed to respond to rest in bed, Lugol's solution and x-ray therapy, complete ablation of the thyroid gland was performed. Following this procedure the metabolic rate dropped strikingly, the clinical signs and symptoms of hypermetabolism disappeared as did the signs of circulatory failure. The patient gained weight, and the lymph nodes and spleen regressed about 90 per cent from their original size. There was complete disappearance of all the patient's symptoms, and the blood picture became almost normal. Dameshek[20] reports that she is "continuing to be healthy" seventeen months after the operation. This amazing result commands attention and invites further clinical trial cautiously carried out.

Thyroid Gland and Lymphocytosis.—Gottlieb[1] in studying the differential counts of blood taken from the thyroid vein of six cases of hyperthyroidism, noted the lymphocytic ratio was increased 33 per cent when compared with that taken from the blood of the thyroid artery and capillary bed. This observation seemed to indicate that a large number of lymphocytes were added to the blood as it circulated through the thyroid gland. Considering the findings of the pathologists that in hyperthyroidism there is a general lymphatic hyperplasia with lymphatic foci in the thyroid gland itself, Gottlieb assumed that this increase of the lymphocytes in the thyroid vein must have been due to an actual formation of lymphocytes in the thyroid gland. However, Menkin[11] felt that the reason for the relative lymphocytosis in hyperthyroidism was due to a sympathetic stimulation of the lymphoid tissues, particularly to the contraction of the spleen, which caused a discharge of lymphocytes into the blood. It has been shown by Harvey[21] and Menkin[22] that contraction of the spleen by the use of drugs or emotional excitement, may increase the lymphocytes of the blood. In our minds, the reason for the lymphocytosis in hyperthyroidism has not been answered, and we are now studying this problem but have not, as yet, formed any definite conclusions.

Thyroid Gland and Anemia.—Anemia associated with myxedema is a well known clinical observation that has received much comment in the literature.[23-27] The classification of the anemia and its treatment has been the cause of considerable variance of opinion. Lerman and Means[23] reported that 50 per cent of their patients with myxedema have a gastric anacidity, and that anemia is much more common in that group than in those showing free acid. Although the anemia improved in a few instances as the myxedema was treated, they urged the addition of iron in adequate doses to bring about a more rapid improvement in the blood. Stone[26] felt the anemia was the result of a depression of erythropoiesis which in turn is a part of a generally diminished function shared by the other tissues. MacKenzie[24] concurs with the belief that the anemia is simply a manifestation of the effect on the bone marrow of the sluggish oxidation that occurs in all tissues.

EXPERIMENTAL INVESTIGATION

Problem.—With these clinical facts and observations in mind, and with comparatively few references to investigative work in this field, we made the following studies. We felt that a complete removal of the thyroid gland in ani-

mals would give us information as to (1) the occurrence and type of anemia that develops in myxedema, and (2) the effect of total ablation of the gland on the normal differential count.

Experimental Procedure.—Four albino rabbits six months old were subjected to a complete thyroidectomy, under ether anesthesia. No signs of infection or tetany developed during the postoperative course. A control series consisted of four rabbits of the same age and weight, two of which were subjected to ether anesthesia and a simple incision of the neck exposing but leaving the thyroid gland intact. This was done in order to insure against any immediate effect on the blood that might arise from either the general anesthesia or surgical incision. Control and thyroidectomized animals were kept together in the same

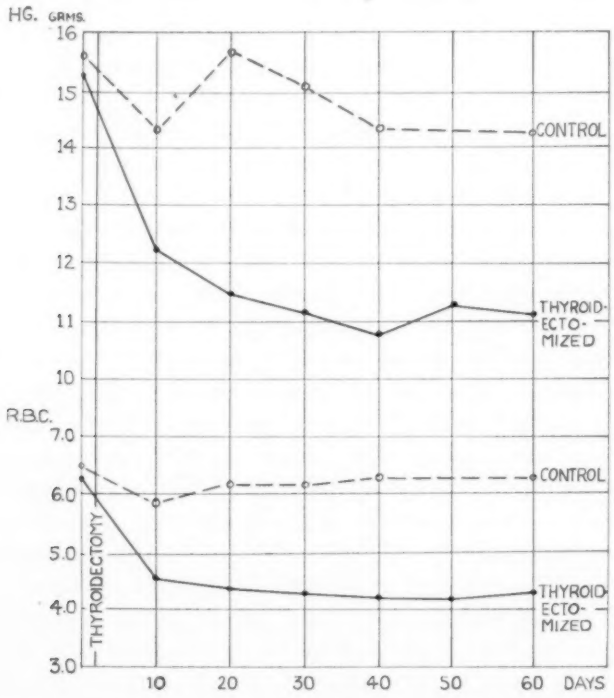

Fig. 1.—Hemoglobin and red cell counts of normal and thyroidectomized animals.

pens and on the same standard diet. Weekly records were kept of their weight. Blood counts were done before and at ten-day intervals following the operation. The blood was taken from the ear, during fasting periods of constant length, and at the same time of day, in order to minimize any error that might result from the normal rhythm of the leucocytes.[28] The rabbits were accustomed to being handled, and made little or no resistance.[22] Hemoglobin determinations were done by means of a Sahli hemoglobinometer (17 gm. equals 100 per cent), and standardized pipettes were used in the determination of the number of the red and white blood cells. Fixed smears were taken on cover slips, stained by Wright's method, and a hundred cells counted on each of a pair of cover slips. Hematocrit determinations were done with the Wintrobe tubes, using the method as devised by him.[29, 30]

Experimental Observations.—During the first ten-day period following operation, there was a definite, sharp drop in both the hemoglobin and red blood cells (Fig. 1). A further, but slower, decrease continued for the first month, after which time the hemoglobin and red blood count remained at about the same level. The control series showed only a slight decrease in the hemoglobin and red cell count, and after the first ten-day interval, both returned and remained within normal limits.

These observations are consistent with the findings of Esser,[31] Kishi,[32] and Mansfield.[33] However, Kunde and his associates,[34] working with much younger rabbits (eretins), did not notice any significant change in the number of red blood cells until four or five weeks following thyroidectomy. After that time, an anemia developed of moderate and persistent degree. Their findings agree with

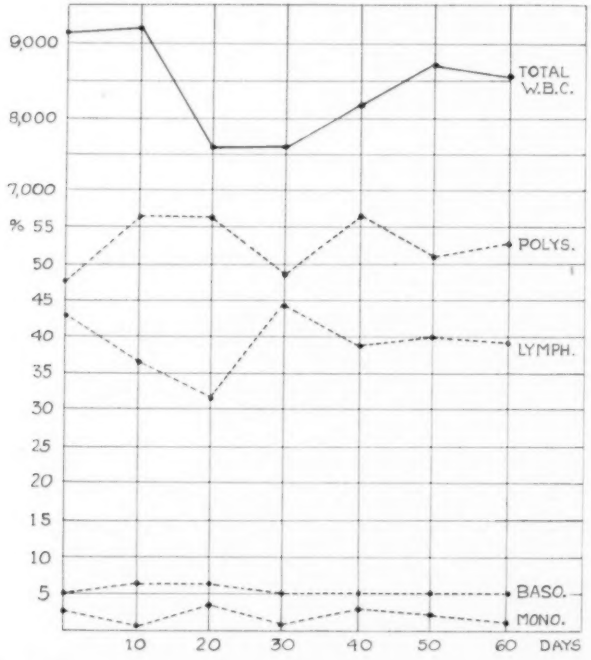

Fig. 2.—White blood cell and differential counts of thyroidectomized animals.

ours, however, in stating that the anemia is characterized by a color index of plus-one and over, and a red cell that is larger than normal. The results of our hematocrit readings are tabulated in Table I. Our normal readings check closely with Wintrobe's observations on rabbit's blood.[35] It is interesting to note that the size of the normal red cell of the rabbit is only about three-fourths that of the human red cell. In the thyroidectomized group, there is a definite increase in the mean corpuscular volume; there is a very slight increase in the mean corpuscular hemoglobin; and no change in the mean corpuscular hemoglobin concentration. These findings are characteristic of a macrocytic anemia.

The number of white cells showed a delayed, slight reduction that reached its lowest level during the second to third week, but remained within normal limits (Fig. 2). A study of the differential formula during each ten-day interval

TABLE I

HEMATOCRIT READINGS

	NORMAL		THYROIDECTOMIZED
	WINTROBE	AUTHORS	
Hemoglobin, grams	13.02	14.7	11.2
Red blood cells, per c.mm.	6.29	6.15	4.38
Volume packed red cells, c.c.	39.79	38.80	30.50
Mean corpuscular volume, c. microns	63.70	61.70	70.50
Mean corpuscular hemoglobin, micromicrograms	21.00	23.50	26.50
Mean corpuscular hemoglobin concentration, per cent	33.20	38.10	37.00
Sedimentation rate, mm./hr.		1.0	1.0

revealed, except for slight minor fluctuations, no significant change from the normal.

The sedimentation rate of the red cells of the rabbit is very much slower than in the human being. The normal rate of a control series was 1 mm. per hour. There was no change in the sedimentation rate of the thyroidectomized animals.

There was a steady gain in weight of the myxedematous animals, and with the exception of some loss of hair, and a definite decrease of activity, the animals appeared to be otherwise healthy.

In addition to the hematologic observations, complete studies of the blood chemistry were followed, and these, with studies of the effect of thyroid feeding on the anemia, and with final pathologic studies of the spleen, lymph nodes, and bone marrow, will be reported at a later date.

SUMMARY

1. Hematologic observations are reported on four normal rabbits and four rabbits in which a complete thyroidectomy had been performed.

2. A moderate and persistent anemia rapidly developed after the removal of the thyroid gland. The anemia is of the macrocytic type.

3. There is a very slight, transient decrease of the white blood cell count; no significant change in the differential formula.

4. There is no change in the sedimentation rate following thyroidectomy.

We wish to thank Miss Mildred Braden for her very valuable technical assistance.

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ATTEMPTS TO APPLY THE ACETYLENE METHOD OF DETERMINING THE CARDIAC OUTPUT TO THE DOG*

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IT HAS not been a great many years since estimations of human blood pressure first came into use as an important criterion of the circulatory state. More recently it has become possible to determine accurately the amount of blood expelled by the heart. The various methods now available for the determination of the cardiac output undoubtedly will advance the understanding of circulatory problems quite as much as did the use of the sphygmomanometer. These methods for the determination of cardiac output are no longer simply laboratory procedures; they are used in the clinic by both internist and surgeon.

In man the Grollman acetylene method[1, 2] gives remarkably constant values for the output of the heart under any constant condition. In experimental animals the Fick method is commonly used to determine the cardiac output. This method, however, not infrequently gives wide fluctuations in the results when numerous repeated determinations are desired. These fluctuations seem to be inherent in the method. Perhaps the puncture of the heart with its disturbance to the rhythm of the heart beat produces some of the deviations, but the withdrawal of a mixed sample of arterial and venous blood is the common source of error. This paper records the attempts to apply the acetylene method of determining the cardiac output to the dog.

Certain conditions must be fulfilled in order to obtain reliable results with the acetylene method. It must be possible to produce a homogeneous mixture between the air-acetylene mixture in the gas container and in the alveoli. There must be perfect equilibrium between the acetylene tension in the alveolar air and in the blood passing through the lungs at the time of taking samples of the alveolar air. Two adequately spaced alveolar samples must be secured before the completion of a single circulation of the blood.

Difficulty was encountered in having the dog breathe so that satisfactory mixtures of the gases could be obtained. Several unsuccessful attempts were made to force the dog to breathe deeply and quickly from a spirometer by stimulation of the saphenous or sciatic nerves. A mechanical method of respiration was then devised by which the gas mixture was forced back and forth between a bag and the alveoli through a tube in the dog's trachea.

The container for the gas mixture is illustrated in Fig. 1. The top (*a*) and base (*b*) of the container were cylindrical pieces of wood 13 cm. in diameter, around which was fitted heavy rubber to form the body of the bag (*c*). From opposite sides of the base were two openings to the outside, (*d*) to admit the gas mixture and (*e*) to connect with a three-way aluminum valve (*f*). A coil spring (*g*) on the inside kept the bag in the expanded position

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unless forced down by the handle (*h*). Copper capillary tubing (*i*) lead to sampling tubes for collection of alveolar air. All connections were hermetically cemented. The bag held approximately one liter of air.

The three-way valve (*f*, Fig. 1) was, in turn, attached by air-tight connections to a tube in the dog's trachea. In the sacrifice experiments a large, heavy-walled rubber tube was used and a ligature passed around the trachea. In the survival experiments a metal tube (*a*, Fig. 2) 12 mm. in diameter was found to be satisfactory. A copper capillary tube (*b*) inside the metal tube (*a*) provided a means of inflating by a pressure bulb (*c*) a piece of a finger

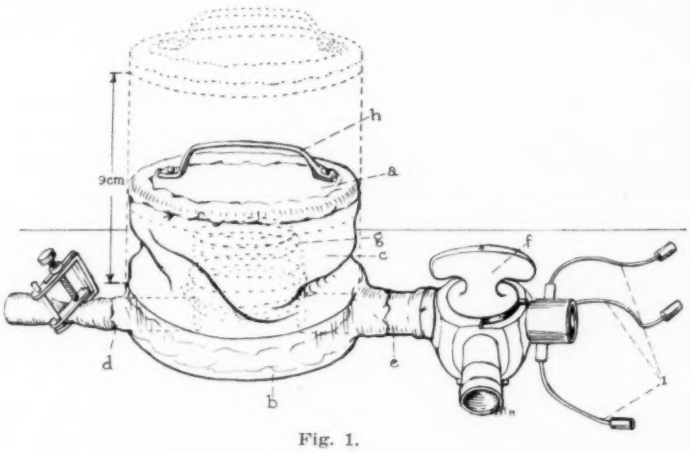

Fig. 1.

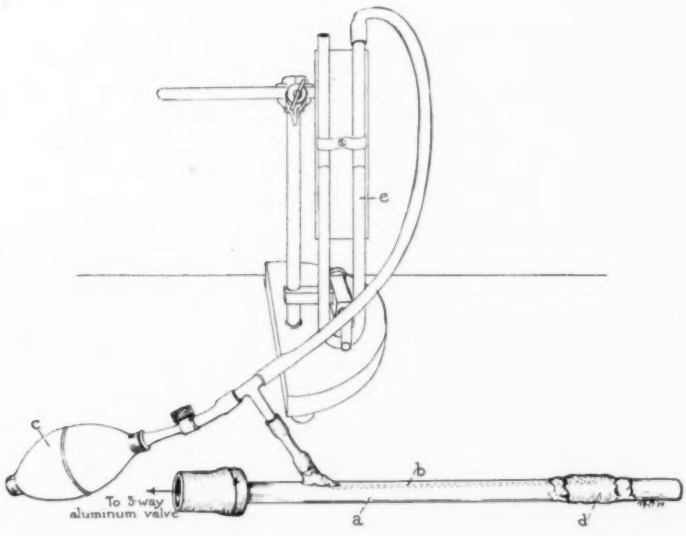

Fig. 2.

from a surgical rubber glove (*d*) which was tied and hermetically sealed around the large tube, so that no air could escape around the tracheal tube, an absolutely necessary condition. A mercury manometer (*e*) was inserted so that any leakage might be instantly detected.

In order to determine the time necessary to establish equilibrium between the acetylene in the alveolar air and in the blood, simultaneous blood and alveolar samples were taken at various intervals after the beginning of rebreathing the air-acetylene mixture. The carotid artery was cannulated and the blood samples

were collected over mercury in an air-tight chamber coated with potassium oxalate. Alveolar samples were collected in evacuated sampling tubes. Alveolar samples were analyzed for acetylene tension on the Haldane gas analysis apparatus (11 c.c. burette) as described by Grollman[2] and blood samples were analyzed for acetylene tension on the Van Slyke apparatus by the method described by Grollman, Proger and Dennig.[3] Since there is some error inherent in the method of analysis, particularly of the gas sample, equilibrium was considered to have been established when the tensions in the simultaneous blood and alveolar samples agreed within 10 per cent.

When the samples were taken exactly fifteen seconds after the beginning of rebreathing the gas mixture, in 7 out of 9 experiments the acetylene tension in the blood and lungs agreed within 10 per cent; there was agreement within 10 per cent in 7 of 9 experiments when the samples were taken after more than 15 seconds, namely, at 16, 17, 18, 19, 20, 23 and 28 seconds; but in samples obtained under 15 seconds, namely 10, 12, 13 and 14 seconds, there was agreement within 10 per cent in only 3 out of 9 experiments.

Our data indicate that by fifteen seconds from the beginning of rebreathing the acetylene tensions in the alveoli and blood are usually in equilibrium. There may be equilibrium in some instances by fourteen or even thirteen seconds from the beginning of rebreathing; but, since it is not possible to predict when this will be true, it is not safe to collect the first sample before the fifteenth second after the beginning of rebreathing.

Attempts were made to obtain equilibrium more quickly by increasing the rate of the rebreathing. Although we do not have sufficient data on this point to justify definite conclusions, our results lead to the belief that the optimal rate is one complete respiration approximately every two seconds.

As noted above, it is essential that the final sample be procured before the completion of a single circulation of the blood. Starr and Collins[4] have found that in the dog an average of 35 per cent of the blood put out by the left heart has returned to the right heart within fifteen seconds. It would seem improbable, therefore, that a correct value for the output would be obtained from calculations based upon the difference in gas content of two samples, one of which was procured fifteen seconds and the other more than fifteen seconds (twenty-two to twenty-eight seconds) after the beginning of rebreathing.

In five different dogs, the results of cardiac output tests obtained by the acetylene method were compared with the results obtained by the direct Fick method. In all of our experiments the dogs were in a basal condition. After determination of the oxygen consumption, the heart was immediately punctured for blood samples for the Fick test, and usually within fifteen minutes (twenty-five minutes in one test) the indirect test was carried out.

Fig. 3 shows that in every instance the output computed by the acetylene method is much too low. The output, as determined by the acetylene method was 32 per cent less in one dog, 48 per cent less in another, and more than 50 per cent less in the other three than the results obtained by the direct Fick method indicate.

On three dogs, under morphine plus pentobarbital anesthesia, series of tests by the acetylene method were carried out on different days. There was a wide variation in the output values obtained for each dog, although the animal was in as nearly identical condition (basal) as possible for all tests. For example, in one case there was a 28 per cent difference between the largest and the smallest values obtained for the minute output; in another instance there was a 35 per cent difference and in another a 30 per cent difference between the two extreme values.

The cardiac indices obtained on these animals (the cardiac output per minute per square meter of body surface) also show great variation, and they are

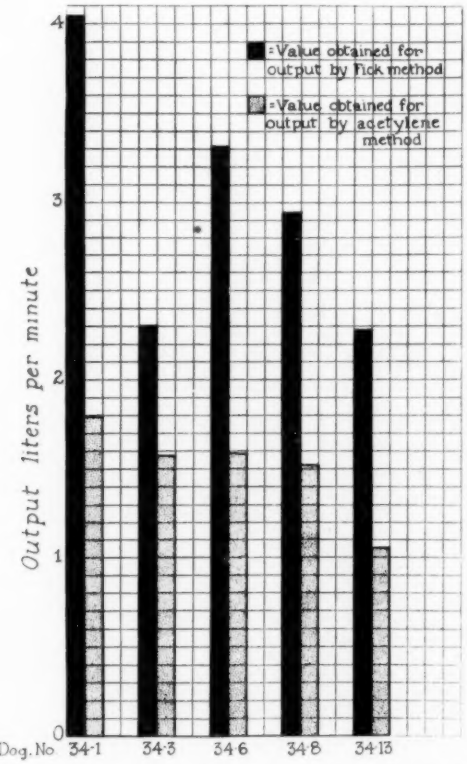

Fig. 3.—Comparison of values for the cardiac output of the dog by the Fick and by the acetylene methods.

much too low. One of us (Williams), using the Fick method, has calculated the cardiac index on a series of seventeen normal dogs and found it to average 3.95. The average value obtained on our dogs by the acetylene method is only 2.15 (average 46 per cent too low).

SUMMARY

Simultaneous blood and alveolar samples taken from the dog at various intervals after the beginning of forced rebreathing of an air-acetylene mixture show that a requisite equilibrium between the acetylene tension in the alveolar air and in the blood can be attained. As a rule, such equilibrium was not estab-

lished before fifteen seconds of rebreathing at the approximate rate of a complete respiration every two seconds. This fact is not favorable to the acetylene method, since a second requisite for its validity is the procuring of at least two adequately spaced samples before the completion of one circulation, and since investigations have been reported (Starr and Collins[4]) indicating return of blood to the heart in such a short period (fifteen seconds) that obviously it would be impossible to obtain two samples within such a period if the first sample is not secured before the fifteenth second.

A comparison of the values obtained for the cardiac output of five dogs by the acetylene method with the values obtained by the direct Fick method shows that the results obtained by the acetylene method are nearly 32 per cent too low in one case and about 50 per cent too low in each of the other four cases.

Tests on the same dog by the acetylene method give wide variation in results, which would preclude its use for comparison of output values before and after production of a circulatory disturbance.

Comparison of the average cardiac index calculated from the acetylene tests (2.15) with the average found in a series of normal dogs by the Fick method (3.95) indicates that the index by the acetylene method is much too low (average 46 per cent).

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THE PREVENTION OF THE ANAPHYLACTIC SHOCK DUE TO HORSE SERUM BY THE INJECTION OF B.C.G.*

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IT HAS previously been shown by one of us (E. M. F.[1, 2]) that bronchial asthma was associated with pulmonary tuberculosis in about 15 per cent of the series studied. Similar reports have been made by other workers in this field.[3, 4] That the incidence of tuberculosis of the lungs is lower among asthmatic individuals than among other groups of subjects has been frequently emphasized,[5, 6, 7] but we cannot agree with the view that has been put forward by other workers that there is a fundamental antagonism between the two conditions. The nonspecific desensitization with small doses of Old Tuberculin for the treatment of asthma and other allergic conditions as proposed by Storm van Leeuwen is suggestive in this connection.

It was shown by Seligmann[8] (1912), W. Pagel and Garcia-Frias[9] (1930), Friedberger and Gajzago[10] (1930), and W. Pagel[11] (1931) that tuberculosis infection in guinea pigs provides protection against anaphylactic shock, and it was demonstrated by W. Pagel and Garcia-Frias that this is not due to a specific tuberculous allergy. This protection is not dependent on the spread of the foci, as subcutaneous injection is sometimes more effective than intravenous injection although the latter provides a greater number of foci. The shock resistance was also transmissible from one infected animal to one noninfected.

Friedberger and Gajzago found that tuberculous infection in guinea pigs did not afford protection in all cases, but that the result depended upon the shocking dose of protein: small doses, effective in control animals, were ineffective, but larger doses of protein still produced shock. Pagel explains the protection produced by tuberculous infection as being due to a failure of antibody response to the sensitizing dose, since the injection of a serum containing antibody is followed by a diminution of the protective power.

Experimental.—In the experiments reported we have investigated the effect of the injection of Calmette's B.C.G. into sensitized guinea pigs. This seemed of interest and importance from both the clinical and theoretical points of view.

Different series of guinea pigs were sensitized with horse serum, and a few days later treated with B.C.G. before the intravenous injection of serum. The controls were sensitized and later on desensitized with the same dose of the same horse serum, but of course without the intermediate injections of B.C.G. Both the time and dose have been varied as may be seen in the tables. Sensitization was carried out with 0.05 c.c. of horse serum and increasing

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doses of B.C.G. emulsion in saline were injected from the eighth day. After a series of doses (subcutaneous and intraperitoneal) during ten days, the animals were left from two to four weeks, and then desensitized by intravenous doses of fresh horse serum.

TABLE I*

ANIMAL NO.	DATE AND SENSITIZING DOSE	DATE AND B.C.G INJECTIONS	DATE AND DESENSITIZING DOSE	EFFECT
1	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 13, 1933 0.5 c.c. serum	No shock
2	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 13, 1933 0.5 c.c. serum	No real shock
3	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 20, 1933 0.8 c.c. serum	No shock
4	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 20, 1933 0.8 c.c. serum	No shock
Control 1	May 2, 1933 0.02 c.c. serum	—	June 2, 1933 0.4 c.c. serum	Shock and death
Control 2	May 2, 1933 0.02 c.c. serum	—	June 5, 1933 0.5 c.c. serum	Shock and death
Control 3	May 2, 1933 0.02 c.c. serum	—	June 13, 1933 0.5 c.c. serum	Shock followed by recovery

*As controls for experimental animals 3 and 4 see Table VII, Nos. 4 and 5; 0.8 c.c. of serum followed by shock and death.

TABLE II

ANIMAL NO.	DATE AND SENSITIZING DOSE	DATE AND B.C.G. INJECTIONS	DATE AND DESENSITIZING DOSE	EFFECT
1	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 10, 1933 0.6 c.c. serum	No shock
2	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 10, 1933 0.6 c.c. serum	Shock and death
3	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 11, 1933 0.8 c.c. serum	Shock and death
4	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 11, 1933 0.8 c.c. serum	No shock
5	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 13, 1933 0.5 c.c. serum	Some convulsions. No typical shock
6	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 13, 1933 0.5 c.c. serum	No shock
Control 1	Sept. 1, 1933 0.02 c.c. serum	—	Oct. 10, 1933 0.6 c.c. serum	Shock and death
Control 2	Sept. 1, 1933 0.02 c.c. serum	—	Oct. 11, 1933 0.8 c.c. serum	Shock and death
Control 3	Sept. 1, 1933 0.02 c.c. serum	—	Oct. 13, 1933 0.5 c.c. serum	Shock and death

Table I.—Three of the animals infected with B.C.G. developed no shock and one, although definitely disturbed, gave no real signs of anaphylactic shock. The controls, injected on the same days as the infected animals with the same doses of horse serum, gave the typical shock leading to death with the exception of Control 3.

Table II shows a similar experiment in which, however, the shock doses were first used on the controls so that no doubt could be entertained that a lethal dose was being employed. The two B.C.G. animals with shock had the same picture at autopsy as the controls: emphysema of the lungs, dilated heart, and no coagulation of the blood. It thus seems that repeated B.C.G.

TABLE III

ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 C.C. OF HORSE SERUM ON JUNE 6, 1933

ANIMAL NO.	DATE AND B.C.G. INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	July 8 and July 31, 1933 0.05-0.1 mg. B.C.G. 4.1 P. 2 s.c.	July 31, 1933 0.3 c.c. serum intravenously	No shock. Died later from intraperitoneal abscess
2	July 8 and July 31, 1933 0.05-0.1 mg. B.C.G. 4.1 P. 2 s.c.	Aug. 4, 1933 0.5 c.c. serum intravenously	No shock
3	July 8 and July 31, 1933 0.05-0.1 mg. B.C.G. 4.1 P. 2 s.c.	Aug. 14, 1933 0.2 c.c. serum intra-arterial	No shock
4	July 8 and July 31, 1933 0.05-0.1 mg. B.C.G. 4.1 P. 2 s.c.	Aug. 14, 1933 0.2 c.c. serum intra-arterial	Prolonged shock
5	July 8 and July 31, 1933 0.05-0.1 mg. B.C.G. 4.1 P. 2 s.c.	Aug. 14, 1933 0.2 c.c. serum intra-arterial	Prolonged shock

TABLE IV

ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 C.C. OF HORSE SERUM ON JULY 8, 1933

ANIMAL NO.	DATE AND B.C.G. INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	July 4 to 14, 1933 4 I.P. 2 s.c.	July 30, 1933 0.3 c.c. serum intravenously	No shock
2	July 4 to 14, 1933 4 I.P. 2 s.c.	Aug. 4, 1933 0.5 c.c. serum intravenously	No shock
3	July 4 to 14, 1933 4 I.P. 2 s.c.	Aug. 4, 1933 0.5 c.c. serum intravenously	No shock
4	July 4 to 14, 1933 4 I.P. 2 s.c.	Aug. 14, 1933 0.2 c.c. serum intra-arterial	Heavy shock
5	July 4 to 14, 1933 4 I.P. 2 s.c.	Aug. 14, 1933 0.2 c.c. serum intra-arterial	Heavy shock

injections into guinea pigs produce protection against anaphylactic shock in the limits above shown, namely when the desensitizing dose is not far above the lethal quantity.

The following experiments, which were carried out with the assistance of Dr. W. Pagel, were designed to examine the effect of variations of time, sensitizing dose, and injection route (intra-arterial).

Tables III and IV.—We investigated the effect of the time of sensitization (before and after B.C.G. treatment) and of the doses for the sensitizing injection.

TABLE V

CONTROLS FOR TABLES III AND IV
ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 C.C. OF HORSE SERUM ON JUNE 24, 1933

ANIMAL NO.	B.C.G.	DATE AND DESENSITIZING DOSE	EFFECT
1	—	July 31, 1933 0.3 c.c. serum intravenously	Shock and death
2	—	Aug. 4, 1933 0.5 c.c. serum intravenously	Shock followed by recovery
3	—	Aug. 14, 1933 0.5 c.c. serum intravenously	Shock and death
4	—	Aug. 14, 1933 0.5 c.c. serum intravenously	Shock and death

The sensitizing dose taken was 0.2 c.c. of horse serum. The animals were injected with B.C.G. four times subcutaneously and twice intraperitoneally. The animals were desensitized with an intra-arterial injection of horse serum. Three of the animals died after immediate shock and one recovered from a very heavy shock.

The five animals sensitized prior to the injection of B.C.G. (Table III) did not develop a fatal shock, one dying later of an intraperitoneal abscess. Three of these animals were desensitized by means of intra-arterial injection; two of these had a prolonged but not deadly shock.

The other series (Table IV) with B.C.G. injections prior to the sensitization showed in three cases no shock and in two cases a heavy but not deadly shock. It would seem that it is immaterial whether the injections of B.C.G. are commenced before or after sensitization, but that the protection afforded is less efficient when a more effective method of desensitization is used (intra-arterial).

In another experiment we determined whether a single small dose of B.C.G. would afford the same protection against the same shock. Some animals were sensitized prior to the injection of B.C.G. (Table VII) and others subsequent to the B.C.G. (Table VI).

Tables VI and VII.—In both series one guinea pig developed no shock, and in Table VI one guinea pig gave a typical shock but without death. In all other

cases a typical anaphylactic shock with death ensued. It may thus be seen that a single small injection of B.C.G. affords no protection against anaphylactic shock. It is to be observed that in the two injected animals in which shock occurred there was no difference from ordinary shock in the gross symptoms or in the postmortem findings. We must thus consider that as in Friedberger's experiment with tuberculous infection the protection afforded by B.C.G. infection is not complete and may fail in a considerable proportion of cases.

TABLE VI

ALL ANIMALS IN THIS TABLE WERE SENSITIZED WITH 0.2 C.C. OF HORSE SERUM ON MAY 9, 1933

ANIMAL NO.	DATE AND B.C.G. INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	May 2, 1933 0.1 mg. s.c.	May 31, 1933 0.5 c.c. serum intravenously	No shock
2	May 2, 1933 0.1 mg. s.c.	June 16, 1933 0.4 c.c. serum intravenously	Shock and death
3	May 2, 1933 0.1 mg. s.c.	June 16, 1933 0.4 c.c. serum intravenously	Shock followed by recovery

TABLE VII

ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 C.C. OF HORSE SERUM ON MAY 2, 1933

ANIMAL NO.	DATE AND B.C.G. INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	May 9, 1933 0.1 mg. s.c.	May 31, 1933 0.4 c.c. serum intravenously	Shock and death
2	May 9, 1933 0.1 mg. s.c.	June 2, 1933 0.25 c.c. serum intravenously	No shock
3	May 9, 1933 0.1 mg. s.c.	June 5, 1933 0.4 c.c. serum intravenously	Shock and death
4	May 9, 1933 0.1 mg. s.c.	June 20, 1933 0.8 c.c. serum intravenously	Shock and death
5	May 9, 1933 0.1 mg. s.c.	June 20, 1933 0.8 c.c. serum intravenously	Shock and death

In the infected animals which died in shock, postmortem examination showed enlarged lymph glands in the neck and thorax but no other sign of B.C.G. infection.

Some unrecorded experiments have been carried out with B.C.G. cultures which were attenuated, and which produced no local lesions after injection, as well as some experiments with an emulsion of bacilli which had been heated

to 56° C. for thirty minutes. These experiments showed that in this case there was no protection whatsoever against the anaphylactic shock.

Experiments where a local abscess was produced with staphylococci gave no immunity to shock. Some other experiments have been tried with sensitization toward extracts of pollen and goose feathers, but no real sensitization could be obtained with these materials.

CONCLUSIONS

1. Repeated doses of B.C.G. emulsion in saline produce a state in the sensitized animal which protects it from the anaphylactic shock due to horse serum.

2. With the desensitizing or shocking dose much in excess of the fatal dose, the protection becomes less effective.

3. Single small doses before or after sensitization do not induce the state of protection against serum shock.

4. The injection of attenuated or killed bacilli or the production of abscesses with other bacteria does not induce any degree of protection.

We wish to acknowledge the collaboration of Mr. J. W. Walker in this work.

The investigation was commenced in the department of the late Professor Calmette in the Institut Pasteur, Paris. We are indebted to Professor Calmette for facilities for work and for subsequently supplying us with cultures of B.C.G. at the Westminster Hospital where the work was continued with the aid of the John Profit Bequest for Tuberculosis.

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A STUDY OF THE EFFECTS OF VACCINE INJECTIONS UPON SKIN SENSITIVITY*

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CERTAIN changes in the degree of skin sensitivity to intradermal vaccine injections have been seen to occur as a result of vaccine therapy. This paper records some observations of such changes and our ideas regarding their significance. Vaccine skin reactions have been such useful guides to us in diagnosis and treatment that we believe that we are justified in presenting the results of this study. These results appear to us to add support to our opinion that autogenous vaccine skin reactions are significant and specific phenomena.[1]

The types of vaccine skin reactions, as already described by us,[1, 2] are (1) a wheal appearing a few minutes after the intracutaneous injection of a vaccine and (2) a tuberculin type of skin reaction which reaches its maximum development in about forty-eight hours.

1. The early or wheal-areola type of skin reaction differs in no wise from that following the intradermal injection of pollen or other protein extracts in sensitized patients.

2. The late local skin reaction, on the other hand, is produced, read, and interpreted so differently by various observers that confusion exists in regard to its exact nature. In order to assist in interpreting this reaction, we repeat here our conception of it. We have defined it as being a lesion of the skin beginning about six hours after and at the site of the intracutaneous injection of not less than 0.01 c.c. nor more than 0.02 c.c. of a 1 per cent volumetric suspension of killed bacteria in normal saline solution and 0.25 per cent trikresol and persisting for forty-eight hours or more. Such a lesion is usually composed of a palpable nodule overlaid by a deep red macule and surrounded by a pink areola. Tenderness and local heat may be present as well as other features that are named in Table I.

The data upon which this paper is based were obtained from the case histories of thirty-three patients suffering with allergic symptoms associated with infection; in other words, cases of bacterial allergy (Table II). In addition to other examinations of these patients, a bacteriologic survey was made of all of their accessible foci of infection, of all abnormal discharges and, in some cases, of the feces. A vaccine of each recovered organism was prepared according to an established technic and was accurately standardized. The patients were then tested intracutaneously with each autogenous vaccine and afterward received therapeutic injections of those organisms which had produced positive reactions. The duration of vaccine treatment

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varied from about three months to three years. Inoculations were made intracutaneously in testing and either intracutaneously or subintracutaneously in treatment.* In each of these two methods the resulting local reactions are easily observed throughout the course of treatment. The intensity and duration of the late local reactions were used as guides in the selection, not only of the vaccines to be used, but also of the amount of dosage. At some time during or at the end of treatment, new tests were performed in each patient with some of the original vaccines.

TABLE I

FEATURES OF THE LATE LOCAL VACCINE REACTION

NO.	FEATURE	AS RECORDED
1	Nodule	N
2	Redness (macule)	R
3	Areola	A
4	Swelling	S
5	Tenderness	T
6	Pain	Pa
7	Heat	H
8	Ecchymosis (icterus)	E
9	Pruritus	Pr
10	Pustule	P
11	Desquamation	D
12	Pigmentation	Pi
13	Lymphangitis	L
14	Lymphadenitis	G

TABLE II

CLINICAL DIAGNOSES OF THIRTY-THREE PATIENTS

Allergic coryza	5
Allergic coryza and aphthous stomatitis	1
Asthma	12
Asthma and bronchiectasis	2
Asthma and chronic bronchitis	3
Asthma and emphysema	1
Asthma and pollinosis	1
Asthma and urticaria	1
Arthritis (chronic rheumatoid and mixed)	6
Arthritis, rheumatoid, and mucous colitis	1
	33

The changes observed in the skin sensitivity of these patients to some of their autogenous vaccines are recorded in Tables III and IV. In each case, these changes were measured by comparing the results of the original tests with those of the retests. Such changes, when they occurred, are recorded in terms of the early and late local reactions.

One hundred and six tests before treatment and the same number of retests after treatment were made with the 106 vaccines listed in Table III. All of these vaccines were used as treatment material. It is seen that skin sensitivity as manifested by the early local reaction remained unchanged after treatment in 67 per cent, was decreased in 23 per cent, and increased in

*A subintracutaneous injection is one which deposits the vaccine into the deeper layers of the skin and into the subcutaneous tissue immediately adjacent thereto. In making such an injection, the point of the needle, after puncturing the skin, is turned back into the under surface of the skin and at this point the syringe is emptied.

10 per cent. On the other hand, skin sensitivity as manifested by the late local reaction (bacterial allergy) remained unchanged after treatment in 17.9 per cent, was decreased in 72.6 per cent, and increased in 9.5 per cent.

Sixty-two tests and the same number of retests were made with the sixty-two vaccines listed in Table IV. None of these vaccines were used in treatment. As in the case of those vaccines which had been used in treatment, but few changes were noted in skin sensitivity of the early or wheal-areola producing type. Quite a different state of things is seen, however, in regard to the type of skin sensitivity manifested by the late local reaction. Here, only about 40 per cent of the retests were followed by reactions indicating diminished skin sensitivity in contrast to a regression in skin sensitivity to 72.6 per cent of the vaccines used in treatment (Table III).

Thus it is seen that the early type of skin reactivity to the majority of the vaccines, whether they were used in treatment or not, persisted unchanged after treatment. This fact seemed to bear no relationship to the presence or absence, or to the degree of clinical improvement. The delayed or tuberculin type of skin sensitivity, however, appeared to have been changed in a definite manner following vaccine treatment. Skin tests after treatment with those vaccines which were used in treatment were followed in most cases by reactions which indicated that reduction in bacterial allergy had occurred.* This reduction, moreover, was proportionate to the degree of clinical improvement of the patient at the time of retest. In the case of those vaccines which were not used in treatment, retests were followed by late local reactions which, on the whole, showed no consistent changes in bacterial allergy. These facts are brought out in Tables V and VI which are summaries of Tables III and IV, respectively. Here the thirty-three patients are divided into groups according to the degree of clinical improvement present at the time of retest. The early type of skin reactivity was found to be unchanged after 60 per cent or more of the retests in all groups in both tables. Delayed skin sensitivity was diminished, in the case of the vaccines used in treatment, after 85 per cent of the retests in patients who were completely relieved or markedly improved, after 66 per cent of the retests in those who were moderately improved, and after 38 per cent of the retests in those who had little or no improvement. In contrast to these findings, skin sensitivity, after retests with the vaccines not used in treatment, was found to be either unchanged, decreased or increased in an irregular manner, not consistent with the presence or absence of clinical improvement.

Some of the skin reactions observed in two individuals are represented in graphic form in Charts 1 and 2. The early or wheal reactions to each organism are recorded by sketches approximately the size and shape of the reactions on the patient's skin (see upper part of Chart 1). In cases in which the wheal was surrounded by an areola the fact is indicated by the letter *A* within the sketch. The late local reactions are represented in a quantitative manner by block graphs, in which each block stands for a separate feature or component part of the reaction (see Chart 2 and the lower part of Chart 1). At the left side of each section of the groups of blocks is a letter identifying

*Since this paper was written Wainwright has reported similar observations (Wainwright, Chas. W.: The Treatment of Chronic Rheumatoid Arthritis with Streptococcus Vaccine, on the Basis of Skin Sensitivity, J. A. M. A. **103**: 1357, 1934).

TABLE III

CHANGES IN THE SKIN SENSITIVITY OF THIRTY-THREE PATIENTS TO THE VACCINES WITH WHICH THEY WERE TREATED, EXPRESSED IN TERMS OF THE EARLY AND LATE LOCAL REACTIONS

PATIENT	CONDITION AT TIME OF RETEST	VACCINES USED IN TESTS AND IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
1 S. A.	Markedly improved	Atypical gram neg. coccus	U*	D†
		Streptococcus viridans	U	D
2 A. H.	Moderately improved	*Staphylococcus aureus*	U	D
		Staphylococcus aureus hem.	I‡	D
3 B. M.	Markedly improved	*B. coli communior*	U	D
		Micrococcus catarrhalis	U	D
		Streptococcus hemolyticus	U	D
		B. coli communis	U	U
4 R. P.	Moderately improved	*Staphylococcus albus*	I	U
		Streptococcus hemolyticus	U	D
		Streptococcus viridans	U	U
5 C. B.	Completely relieved	*Micrococcus catarrhalis*	U	U
		Staphylococcus albus	D	D
		Staphylococcus albus hem.	D	D
		Streptococcus viridans	U	D
6 J. S.	Completely relieved	*B. alkaligines*	I	D
		Staphylococcus aureus	U	D
		Streptococcus viridans	U	D
		Streptococcus viridans	D	D
7 A. W.	Slightly improved	*Micrococcus albus*	U	D
		Staphylococcus aureus	U	D
		Streptococcus hemolyticus	U	D
		Streptococcus viridans	D	D
8 A. V.	Markedly improved	*Streptococcus viridans*	U	D
		Streptococcus hemolyticus	I	D
		Micrococcus catarrhalis	U	D
		B. coli communis	U	D
		Staphylococcus aureus	U	D
9 B. G.	Markedly improved	*B. coli communior*	U	U
		Streptococcus hemolyticus	U	D
10 C. C.	Markedly improved	*Staphylococcus albus*	U	D
		Streptococcus viridans	U	D
23 A. B.	Moderately improved	*B. coli communis*	U	U
		Micrococcus catarrhalis	U	D
		Enterococcus indifferens	U	D
		Staphylococcus albus	U	D
		Staphylococcus aureus	D	D
24 M. V.	Unimproved	*B. coli communis*	U	I
		Staphylococcus aureus	U	D
		Staphylococcus albus	U	D
25 J. S.	Slightly improved	Atypical gram neg. coccus	U	D
		B. alkaligines	D	D
		B. morgani	I	U
		Streptococcus hemolyticus	I	U
26 R. S.	Completely relieved	*B. coli communior*	D	D
		B. coli communis	D	D
		B. coli communis hem.	D	D

*Unchanged. †Diminished. ‡Increased.

TABLE III—CONT'D

PATIENT	CONDITION AT TIME OF RETEST	VACCINES USED IN TESTS AND IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
27 E. S.	Markedly improved	*Staphylococcus albus*	D	D
		Staphylococcus albus Hem.	D	I
		Streptococcus viridans	U	D
28 F. P.	Unimproved	*Micrococcus catarrhalis*	U	I
		Staphylococcus aureus	U	D
		Streptococcus indefinite	U	D
29 E. P.	Markedly improved	*B. coli communis*	U	U
		B. coli communis hem.	U	U
		Enterococcus indifferens	U	D
30 H. W.	Completely relieved	*B. coli communior*	U	D
		B. coli communior hem.	D	D
		Enterococcus viridans	D	D
31 C. W.	Completely relieved	*B. coli communis*	U	D
		Streptococcus indifferens	D	D
		Streptococcus indifferens	D	D
32 E. S.	Markedly improved	*B. coli communis*	U	U
		B. coli communis hem.	U	D
		Enterococcus hemolyticus	U	D
		Enterococcus viridans	U	D
33 J. McC.	Completely relieved	*B. coli communis*	I	D
		B. coli communis hem.	U	D
		Streptococcus hemolyticus	D	D
		Streptococcus viridans	D	D

Reactions:	Unchanged	Decreased	Increased
Early local	71 (67.0%)	24 (23.0%)	11 (10.0%)
Late local	19 (17.9%)	77 (72.6%)	10 (9.5%)

that section as the representative of one of these features. Reference to Table I will assist the reader in understanding these graphs. There are listed the features or components observed as parts of the late local reaction. These bear the initial letters of their names as symbols in our usual method of recording their presence in reactions.[1] Repetition of a symbol two or more times signifies that the feature represented is two or more times larger than the minimum size. In like manner, in the graphs, the amount of horizontal extension of a block indicates the quantity of a separate feature. The transverse lines drawn between the N-R groups of blocks and those blocks above them indicate a separation of the nodule-macule from all other features of the reaction. We have evidence which suggests that the nodule-macule portion of the reaction is associated with an immunity mechanism separate from that of bacterial allergy of which the other components are manifestations.[4] The late local reactions seen before treatment and those seen after treatment are recorded in both of the charts; the late local reactions observed during the course of treatment are also recorded in Chart 2. The number of vaccine doses and the total amount of vaccine substance, in milliunits (MU),* received by each patient as well as other pertinent data are also recorded.

*We have used the arbitrary standards of vaccine units and milliunits as measures of quantity of bacterial substance in testing and treatment vaccines. The *Vaccine unit* is 0.01 c.c. of a 1 per cent volumetric suspension of killed bacteria and the *milliunit* is 0.001 of a unit.[5]

TABLE IV

CHANGES IN THE SKIN SENSITIVITY OF TWENTY-FOUR PATIENTS TO VACCINES WHICH WERE NOT USED IN TREATMENT. THE CHANGES ARE EXPRESSED IN TERMS OF THE EARLY AND LATE LOCAL REACTIONS

PATIENT	CONDITION AT THE TIME OF RETEST	VACCINES USED IN TESTS BUT NOT IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
3 B. B.	Markedly improved	*B. coli communis*	U*	D†
		Staphylococcus albus	U	D
		Streptococcus viridans	U	U
		Streptococcus viridans	I‡	U
		Streptococcus viridans	U	U
		Streptococcus hemolyticus	U	U
4 R. P.	Moderately improved	Atypical gram neg. coccus	U	D
		Streptococcus indifferens	U	U
5 C. B.	Completely relieved	*Micrococcus catarrhalis*	U	D
		Streptococcus hemolyticus	U	D
		Streptococcus viridans	U	D
7 A. W.	Slightly improved	*Streptococcus indifferens*	U	D
		Streptococcus hemolyticus	U	U
		Micrococcus albus	U	U
8 A. V.	Markedly improved	*Streptococcus viridans*	U	I
		B. coli communior	U	D
9 B. G.	Markedly improved	*Micrococcus catarrhalis*	U	U
		Streptococcus indifferens	U	U
		Streptococcus viridans	U	D
10 C. C.	Markedly improved	*Staphylococcus albus*	U	U
		Streptococcus indifferens	U	D
11 C. R.	Moderately improved	*Streptococcus viridans*	I	D
		Staphylococcus aureus	U	I
		Staphylococcus albus	U	U
		Staphylococcus albus	D	I
12 A. H.	Completely relieved	*Streptococcus viridans*	U	U
13 B. S.	Slightly improved	*Staphylococcus aureus*	I	I
		Streptococcus viridans	I	U
15 A. W.	Completely relieved	*Staphylococcus aureus*	U	D
		Streptococcus hemolyticus	U	D
		Streptococcus viridans	U	I
		Streptococcus viridans	U	I
16 C. M.	Completely relieved	*Staphylococcus albus*	U	I
		Streptococcus hemolyticus	D	U
		Streptococcus viridans	D	I
		Staphylococcus albus	D	D
17 T. D.	Moderately improved	Chromogenic coccus 6	I	D
		Chromogenic coccus 6	U	D
18 W. S.	Markedly improved	*Streptococcus viridans*	U	D
22 F. J.	Moderately improved	*Streptococcus hemolyticus*	I	U
		Streptococcus viridans	U	U
24 M. V.	Unimproved	*Staphylococcus aureus*	U	U
		Streptococcus hemolyticus	U	I
25 J. S.	Slightly improved	*Staphylococcus albus*	U	U
		Staphylococcus albus	D	U
		B. diphtheroid	U	U

*Unchanged. †Diminished. ‡Increased.

TABLE IV—CONT'D

PATIENT		CONDITION AT THE TIME OF RETEST	VACCINES USED IN TESTS BUT NOT IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
26	R. S.	Completely relieved	*Enterococcus indifferens*	U	U
27	E. S.	Markedly improved	*Staphylococcus albus*	D	I
			Chromogenic coccus 6	I	I
			Streptococcus indifferens	U	D
			Streptococcus indifferens	U	I
			Streptococcus viridans	U	I
28	F. P.	Unimproved	*Staphylococcus albus*	U	D
29	E. P.	Markedly improved	*Enterococcus hemolyticus*	U	D
			Enterococcus viridans	U	D
30	H. W.	Completely relieved	*B. coli communis*	D	D
31	C. W.	Completely relieved	*Enterococcus viridans*	U	D
			Atypical gram. neg. coccus	U	U
			Staphylococcus albus	D	D
			Streptococcus hemolyticus	U	U
			Streptococcus viridans	U	U
33	J. McC.	Completely relieved	*Streptococcus hemolyticus*	U	D

Reactions:	Unchanged	Decreased	Increased
Early local	46 (74.2%)	8 (12.9%)	8 (12.9%)
Late local	24 (38.7%)	25 (40.4%)	13 (20.9%)

TABLE V

SUMMARY OF CHANGES NOTED IN SKIN SENSITIVITY OF THIRTY-THREE PATIENTS TO THE AUTOGENOUS VACCINES WITH WHICH THEY WERE TREATED

PATIENTS		NO. OF VACCINES	CHANGES IN SKIN SENSITIVITY AS MANIFESTED BY					
			EARLY LOCAL REACTION			LATE LOCAL REACTION		
NO.	CLINICAL RESULT		UNCHG.	DECR.	INCR.	UNCHG.	DECR.	INCR.
21	Completely relieved or markedly improved	67	45 67.1%	18 26.9%	4 6.0%	7 10.5%	57 85.1%	3 4.5%
6	Moderately improved	18	12 66.7%	4 22.2%	2 11.1%	4 22.2%	12 66.6%	2 11.1%
6	Slightly improved or unimproved	21	14 66.7%	2 9.5%	5 23.8%	8 38.1%	8 38.1%	5 23.8%

TABLE VI

SUMMARY OF CHANGES NOTED IN SKIN SENSITIVITY OF TWENTY-FOUR PATIENTS TO SOME OF THE AUTOGENOUS VACCINES WHICH WERE NOT USED IN TREATMENT

PATIENTS		NO. OF VACCINES	CHANGES IN SKIN SENSITIVITY AS MANIFESTED BY					
			EARLY LOCAL REACTION			LATE LOCAL REACTION		
NO.	CLINICAL RESULT		UNCHG.	DECR.	INCR.	UNCHG.	DECR.	INCR.
15	Completely relieved or markedly improved	41	32 78.0%	6 14.6%	3 7.4%	13 31.7%	19 46.3%	9 22.0%
4	Moderately improved	10	6 60.0%	1 10.0%	3 30.0%	4 40.0%	4 40.0%	2 20.0%
5	Slightly improved or unimproved	11	8 72.7%	1 9.1%	2 18.2%	7 63.6%	2 18.2%	2 18.2%

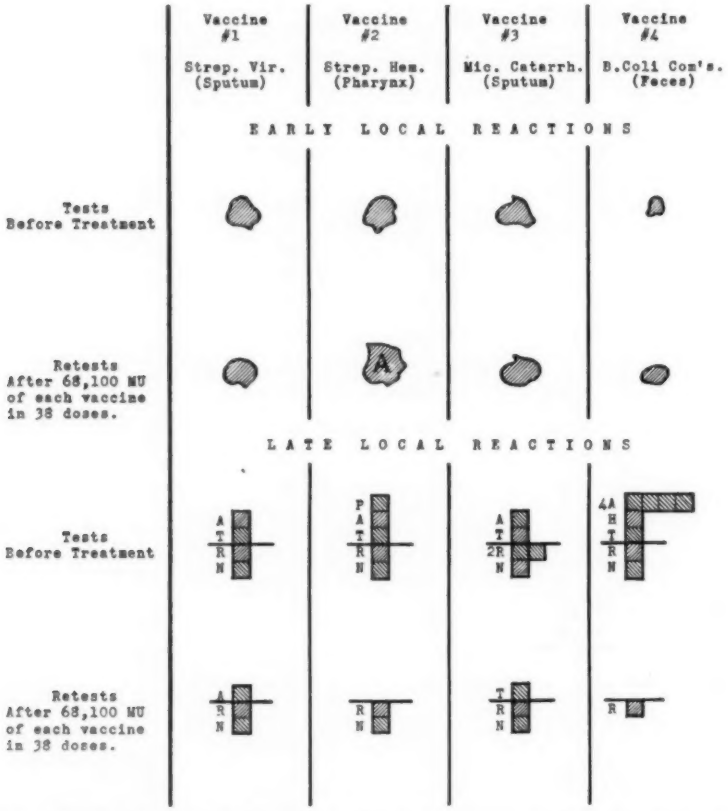

Chart 1.—Graphic representation of early and late local reactions produced by vaccines used in treatment. Patient, A. V. Diagnosis: asthma and urticaria. Duration of treatment: eighteen months. Condition at time of retest: markedly improved.

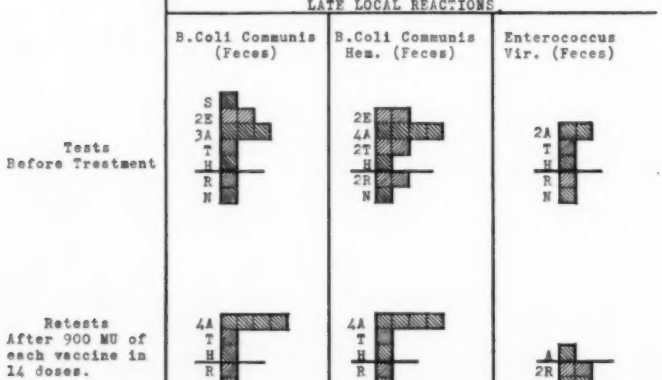
LATE LOCAL REACTIONS
B.Coli Communis (Feces)
B.Coli Communis Hem. (Feces)
Enterococcus Vir. (Feces)
Tests Before Treatment
Retests After 900 MU of each vaccine in 14 doses.
Retests After 5,400 MU of each vaccine in 21 doses.

Chart 2.—Graphic representation of late local reactions produced by vaccines used in treatment. Patient, H. W. Diagnosis: mixed type of arthritis. Duration of treatment: twelve months. Condition at end of treatment: completely relieved.

Chart 1 presents the data of a patient who had asthma and urticaria. After eighteen months of treatment with autogenous vaccines she was markedly improved. A total of 61,000 milliunits of each of four vaccines had been administered in thirty-eight doses. The local reactions following vaccine skin tests with each of these organisms before treatment and those following identical tests after treatment are illustrated. It is seen that there was but little difference between the wheals produced by the several vaccines before treatment and those produced by the same vaccines after treatment. In the case of the late local reactions, on the other hand, regressive changes in skin sensitivity to each of the vaccines were observed.

The patient whose late local reactions are graphically recorded in Chart 2 suffered with chronic arthritis of the mixed type. She received a total of 5,400 milliunits of each of three autogenous vaccines in twenty-one doses. The late local reactions following skin tests with each of these vaccines before treatment and those which followed identical tests during and again after treatment are illustrated. At the time of the first retests, namely six months after treatment was begun, the patient was markedly improved. She had become symptom-free before the second retests were made. Progressive regression in skin sensitivity to all of the vaccines is seen to have occurred in a manner which paralleled diminution in and final disappearance of the symptoms of arthritis.

DISCUSSION

The results of observations in this small series of patients are typical of what has been found in the majority of a large number of patients similarly tested and treated. As we have stated in previous articles,[1, 5] these changes are largely limited to that form of skin sensitivity which is manifested by the late local vaccine reaction and, usually, are regressive in character. An increase in skin sensitivity to one or more organisms has been seen to occur after treatment with mixed vaccines, but never after treatment with single strain vaccines. Other writers have reported increased skin sensitivity following intracutaneous and subcutaneous vaccine injections.[6] Clawson and Wetherby,[7] in a report on intravenous vaccine therapy in arthritis make the following statement: "The subcutaneous method (of vaccine injection) seems to be contraindicated, since it tends to increase hypersensitiveness, does not bring about a state of desensitization and produces only a low degree of protection." It appears to us that an increase in skin sensitivity after vaccine injections occurs only when too large a dose of killed bacteria or some other factor, such as the use of a mixed vaccine, establishes a reservoir of antigenic material in or beneath the skin. Under these circumstances a lesion results which has the effect of a focus of infection. An increase in bacterial allergy may follow if a series of such injections is given.[6] Treatment injections administered by us, however, using vaccine reactions as guides, are adjusted in bulk and concentration so as to produce a minimum of local reaction. Thus the production of focal lesions and an increase in hypersensitivity have usually been avoided. Retests in patients under treatment with mixed vaccines composed of one organism which produced a large late local reaction after the original

skin tests and of others which produced smaller reactions have, at times, revealed increased skin sensitivity to some of these organisms. At other times, it was seen after retests, that reduction in skin sensitivity was proceeding at a more rapid pace in the case of some of the organisms contained in a mixed vaccine than in the case of others. Further injections of the same organisms, but as single strain vaccines, have appeared to rectify these inequalities. The intense local reaction produced by the organism, in a mixed vaccine, to which the patient is most sensitive seems to localize the less potent organisms within the reaction to such an extent that little desensitizing effect can be produced by them. Such a local reaction may even act in a manner similar to that of a focus of infection from which sensitizing doses of the weaker bacteria repeatedly escape.

We would emphasize two observed facts, viz.: (1) not only did a diminution or disappearance of the delayed type of skin sensitivity (to organisms used in treatment) accompany relief of symptoms but also (2) these two synchronous phenomena proceeded in direct proportion to each other.

Collis and Sheldon[8] noted similar results in children with rheumatic fever who received intravenous injections of streptococcus products. They cite case histories "to show that there is a parallelism between clinical improvement, desensitization and the presence of immune bodies in the serum."

The above facts suggest that the symptoms which were relieved may have been allergic manifestations set up by the particular organisms recovered from the patient and used in treatment. Further evidence to this effect exists in the fact that, in the case of patients who were not relieved by treatment (see Table V), the changes in skin sensitivity to organisms used in treatment were almost exactly the same in degree as those observed in patients on retesting with organisms not used in treatment (see Table IV). In other words, so far as specific desensitization to the particular organisms used in treatment is concerned, no greater changes were produced by treatment in unimproved patients than those which would have occurred without treatment.

Persistence of an undrained focus of infection seems to be the usual cause of failure of autogenous vaccine therapy to be followed by improvement of symptoms and by regressive changes in skin sensitivity. Our experience has encouraged us, in these cases, to resume vaccine therapy after such a focus has been found and eradicated.

SUMMARY AND CONCLUSIONS

Early and late local skin reactions following intracutaneous injection of autogenous vaccines are described and defined. Attention is called to certain variations in the reactions which occur when patients are retested during or after courses of vaccine treatment. Data taken from the case histories of thirty-three patients tested and treated with autogenous vaccines are here collected and analyzed in order to demonstrate the following conclusions, namely:

1. Regardless of the degree of clinical improvement following treatment, the early vaccine skin reaction remained relatively unchanged.

2. Regression of the late vaccine skin reaction occurred in the majority of successfully treated patients and failed to occur in most of those receiving no benefit from vaccine treatment.

3. The degree of regression in intensity of the late vaccine skin reaction was in direct proportion to the degree of improvement in the clinical condition under treatment.

4. Vaccine skin reactions, in addition to being useful guides, in the selection of vaccine for treatment, are indicators of the progress of desensitization in vaccine treated patients.

5. Concurrent decrease in the late local vaccine reaction and in the patient's symptoms during vaccine treatment appear to point to a relationship between those symptoms and a specific sensitivity to the organisms used.

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THE NORMAL RANGE OF THE LEUCOCYTE COUNT DETERMINED WEEKLY OVER AN EXTENDED PERIOD*

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THIS study was undertaken to determine the range of the leucocyte count taken weekly in normal subjects under standard conditions. Although the project was an investigation in chronic rheumatic heart disease, it seemed necessary to digress in this direction to decide if possible a normal range, so that the findings in the rheumatic group might be properly correlated.

A perusal of some of the literature revealed many valuable observations as well as many conflicting ones. In some instances investigators have taken isolated counts on large groups, some many counts on small groups, and then again others have noted the influence of many phenomena, such as heat, cold, rest, exercise, and digestion. In their review Sabin, Cunningham, Doan, and Kindwall[1] mention the findings of Galambos, Turk, and Torday, who observed the normal range to be 3,500 to 12,500, 5,000 to 10,000, and 3,130 to 9,800, respectively. Sabin et al.[1] in their study of six normal subjects noted an hourly rhythm of the leucocyte count with a progressive increase in the afternoon, the lowest level being in the morning, whether or not food had been taken. The variation was from 5,000 to 10,000, a ratio of 1:2. The upper limit was 13,000 in one case. Smith and McDowell[2] corroborated these findings and in addition observed no effect of menstruation on the count. Two cases were studied. According to these authors the curves of the daily rhythm had a characteristic pattern for each person. Swift, Miller, and Boots[3] found 8,000-9,000 the upper limit of normal in a group of nonrheumatic persons and patients who had completely recovered from the infection. Their studies were made between eleven and twelve in the morning or three and four in the afternoon. Garrey and Butler[4] determined that at rest the basal count was 5,000 to 6,000, the physical level being 60 per cent to 100 per cent above the basal level. Their observations are in accord with those of Sabin et al.[1] and Smith and McDowell[2] with regard to the fact that there is no digestive leucocytosis.

The unusually wide range of the alleged normal and the varied methods of observation made it necessary to set up a standard method of study. The leucocyte curves of Sabin et al.[1] and those of Smith and McDowell[2] clearly demonstrated that the uniformly low level was the forenoon. The time

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This article is the first of a series to be published on the research investigation at the New York Cardiac Shop, which was a special project of the Committee on Cardiac Clinics of the Heart Committee of the New York Tuberculosis and Health Association. This work was made possible by grants from the Hofheimer Foundation for three years, the New York Foundation for two years, and the Altman Foundation for one year. The balance of the fund was raised by the Board of Directors of the New York Cardiac Shop of which Mrs. Jack Wildberg was the Chairman. This Board was a Subcommittee of the Irvington House of which Mrs. Louis Levy was President.

chosen, therefore, was between 10:30 A.M. and 12 M. on Thursday of each week when possible. No unusual physical exertion was engaged in, and there was no exposure of the hands to extreme heat or cold prior to the procedure, in order to eliminate some of the usual variants. The group consisted of 11

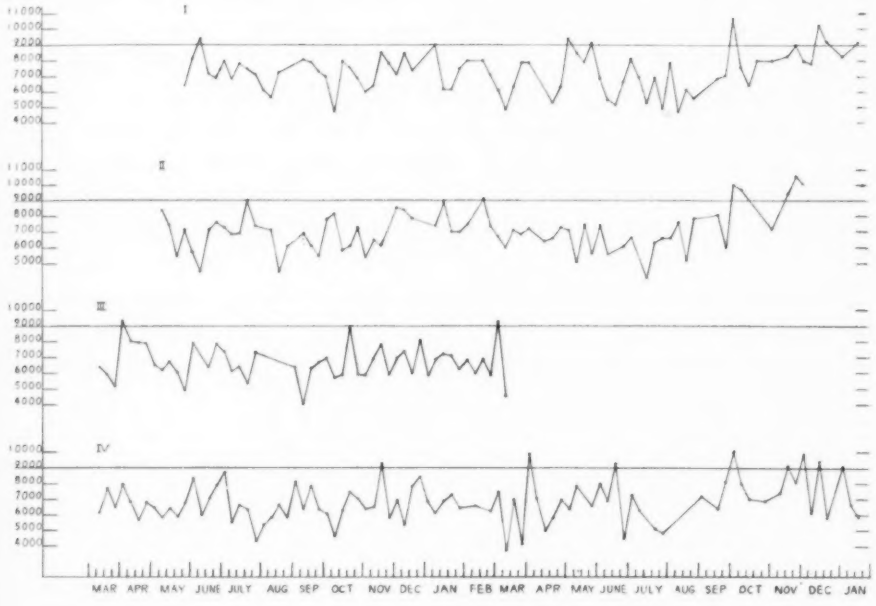

Chart 1.

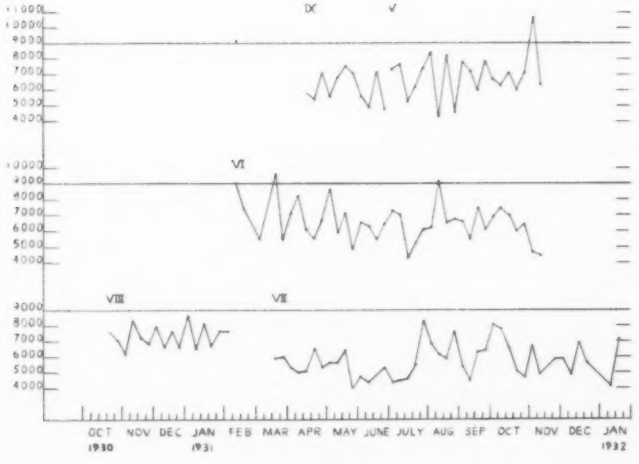

Chart 2.

persons between the ages of seventeen and fifty, 7 females and 4 males, studied for a period from eleven weeks to ninety-eight weeks. The lowest number of counts was 11, the highest 85.

The curves were so strikingly similar in 9 individuals that 2 were excluded as being abnormal, but are also reported. Charts 1 and 2 represent

the leucocyte curves of the nine cases. The ordinates indicate the date of each count and the abscissas the leucocyte count. (Charts 1 and 2.) All the counts were below 11,000 in the upper limit; 435 counts were made, 94 per cent of which were below 9,000. This finding is in agreement with that of Swift, Miller and Boots.[3] All the counts with one exception were above 4,000 in the lower limit. For practical purposes, therefore, the normal range of the leucocyte count is between 4,000 and 9,000. The ratio of 1:2 is similar to that obtained by Sabin et al.[1] and Smith and McDowell[2] in their series.

The curves are practically identical with those of the forenoon of these authors regardless of the fact that the latter were serial determinations on the same day. Furthermore, a careful examination of the morning portions of their curves showed the upper limit to be below 9,000 and the lower limit above 4,000.

It will also be noted in Charts 1 and 2 that season, sex, and age within limits of the group had no influence on the counts. The curves did not show an individual pattern but appeared to fall within 2 groups, 7 in one and 2

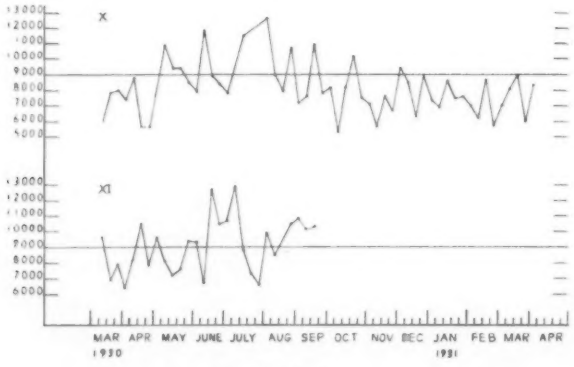

Chart 3.

(Cases 8 and 9) in the other. The latter 2 did not have as wide a range as the former group, the ratio being less than 1:2. This appeared to be the difference between Subject A and Subject B in Smith and McDowell's series.[2] If these authors had studied more than 2 cases perhaps a similar grouping might have been found rather than individual patterns.

Table I shows the number of counts taken on each individual and the percentage below 9,000.

Table II shows the range of counts including the average. It will be noted that the highest average count was 7,300 and that the two abnormals were 8,100 and 9,100, respectively.

Chart 3 shows the leucocyte curves which were considered abnormal. Case 10 has two phases, the first abnormal and the second similar to the curves in the normal group. Case 11 in no way resembles any of the other curves.

There was no recognized physical basis to account for the two abnormal curves. These individuals were in a more emotional state than the others:

the observations of Garrey and Butler[4] who noted leucocytosis in psychologic states might explain these deviations. In addition they were subject to more upper respiratory infections than the rest.

TABLE I

	CASE	TOTAL NUMBER OF COUNTS	PERCENTAGE BELOW 9,000
Normal	1	74	90
	2	70	89
	3	48	96
	4	85	90
	5	20	95
	6	38	94
	7	41	100
	8	16	100
	9	11	100
Abnormal	10	52	77
	11	27	48

TABLE II

	CASE	LOWEST	HIGHEST	AVERAGE
Normal	1	4,700	10,700	7,300
	2	4,200	10,600	7,100
	3	4,000	9,300	6,700
	4	3,700	10,000	6,900
	5	4,300	10,700	7,100
	6	4,300	9,600	6,600
	7	4,000	8,300	5,800
	8	6,200	8,600	7,300
	9	4,800	7,500	6,100
Abnormal	10	5,300	12,600	8,100
	11	6,400	12,900	9,100

SUMMARY

1. The leucocyte counts were determined weekly in eleven normal subjects between the ages of seventeen and fifty. They were studied for a period of eleven to ninety-eight weeks under standard conditions. Counts were taken between 10:30 A.M. and 12 M. once a week, eliminating exposure to heat and cold and avoiding exercise, factors which influence the number of white blood cells.

2. The leucocyte curves of nine persons were so similar that two were excluded as abnormal but are also reported.

3. The curves presented no individual patterns. There appeared, however, to be a possible grouping with seven in one and two in the other.

4. The normal range of the count was 4,000 to 9,000.

5. Sex, season, and age within limits of the group had no influence on the leucocyte count.

CONCLUSION

Under standard conditions the normal range of the leucocyte count is 4,000 to 9,000.

Many thanks are due Doctors Alfred E. Cohn and Homer F. Swift for their most valuable advice and constructive criticism during the period of study; to Mr. Harry Hopkins for his assistance in arranging the budget; and to Doctor Edward Holtz who assisted me during the study.

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THE SIGNIFICANCE OF SERUM INORGANIC SULPHATE CONCENTRATIONS IN BRIGHT'S DISEASE*

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THE physiologic and clinical significance of serum inorganic sulphate concentrations has until recently received little attention. Denis,[1] in 1921, reported a nephelometric method for the determination of inorganic sulphate in blood. She found from 0.5 to 1.0 mg. of sulphate sulphur in the serum of normal persons, with much larger concentrations in the serum of patients with terminal Bright's disease. The well-known technical difficulties inherent in nephelometric procedures have prompted other investigators to develop new methods for serum sulphate. Most of these methods utilize a trichloroacetic acid filtrate from serum and differ from each other merely in the scheme for analyzing the benzidine sulphate precipitated in this filtrate. Though there is a comparatively wide variation in the values obtained by these methods, the results of most of them agree with each other and with those of Denis in two essential features—first, that normal serum inorganic sulphate concentration is of the order of 1 mg. S per 100 c.c., and second, that there is a marked rise in this value as renal inefficiency progresses to uremia in Bright's disease.

Wakefield, Power, and Keith[2] have drawn further conclusions. Reporting on the use of a new oxidimetric method for analyzing benzidine sulphate, they compared serum sulphate concentrations with the results of standard tests for renal function in a large number of individuals. Their cases included normal persons, those with essential hypertension, those with early Bright's disease with and without edema, and those with terminal Bright's disease. These authors have found that in uremia serum sulphate concentration rises along with urea, reaching values of more than 30 times that of normal. In addition, they reported that many patients with hypertension or with early glomerulonephritis, who as yet showed no deviation from the

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normal in most of the common tests for renal efficiency, including that of urea clearance, already showed elevations of serum inorganic sulphate. These authors submit the determination of serum sulphate as a test of early renal inefficiency similar to and as valuable as the concentration test, the phenol-sulphonephthalein test, and the urea clearance estimation.

These results are obviously of great importance if corroborated. Aside from the theoretical physiologic significance of such early rises in serum inorganic sulphate concentration, the practical value of being able to gauge early renal inefficiency by a comparatively simple determination of a blood constituent would be very much appreciated by clinicians. The present investigation was undertaken primarily for the purpose of such a corroboration. But the study has been broadened to include the rôle of serum sulphate in more advanced Bright's disease, especially its effect upon the production of acidosis in uremia.

METHODS

A critical survey was first attempted of the recent methods for determining serum sulphate. It was soon discovered that those methods, including that of Power and Wakefield,[3] which depend upon the precipitation of benzidine sulphate from a trichloroacetic acid filtrate of serum by the addition of benzidine in acetone, are subject to serious errors, because the precipitate obtained is contaminated with other substances, particularly benzidine phosphate. The values obtained by such methods may be too high by about 35 per cent in normal serum and by as much as 300 per cent in serum from uremic patients.

A new method has therefore been developed in this laboratory by Hoffman and Cardon,[4] which attempts to avoid these errors and which gives equally consistent results with both normal serum and that from uremic patients. In this method, a protein-free, phosphate-free filtrate from serum is obtained by coagulating the diluted serum by heat in the presence of ferric chloride, ammonium acetate, and ammonium hydroxide, as in the senior author's[5] method for serum total base. In this water-clear filtrate, sulphate can be precipitated in the usual manner by the addition of benzidine chloride and acetone. Pure satiny crystals of benzidine sulphate are obtained, which, after washing three times with acetone in a specially designed centrifuge tube, are analyzed by oxidation with $KMnO_4$, as in Kramer and Tisdall's[6] method for serum potassium.

The average concentration of serum inorganic sulphate by this method in forty-two normal persons was 0.78 mg. S per 100 c.c. with a standard deviation of ± 0.16 mg. (see Fig. 1). The maximal normal value found was 1.09 mg. S per 100 c.c.

In order to ascertain the rôle of serum sulphate in Bright's disease, a study was made of sixty patients. In these patients, simultaneous determinations were made of serum sulphate, blood urea nitrogen, and the urea clearance. In addition, in those cases of markedly elevated serum sulphate, com-

plete electrolyte balance studies were attempted for the purpose of estimating the part played by sulphate in the production of the acidosis so characteristic of uremia.

The chemical methods employed were the following: urea nitrogen, Van Slyke and Cullen;[7] urea clearance, Moeller, McIntosh and Van Slyke;[8] total base, Hoffman;[5] chloride, Van Slyke and Sendroy;[9] phosphate, Fiske and Subbarow;[10] protein, macro Kjeldahl; carbon dioxide, Van Slyke and Cullen;[11] serum sulphate, Hoffman and Cardon.[4]

The estimation of base combined with phosphate, protein, and bicarbonate was made from data given by Peters and Van Slyke,[12] assuming a serum pH of 7.35. This of course involves an error; but the extent of the error for the total anions probably does not exceed 2 milliequivalents. For the purpose of electrolyte balance studies, an error of such magnitude is insignificant.

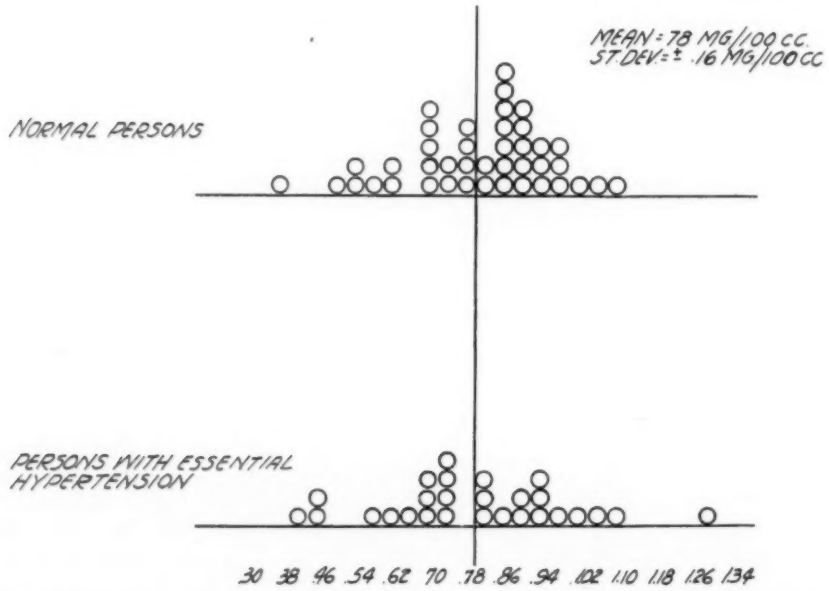
MEAN = 78 MG/100 CC.
ST. DEV. = ± .16 MG/100 CC.
NORMAL PERSONS
PERSONS WITH ESSENTIAL HYPERTENSION
30 38 46 54 62 70 78 86 94 102 110 118 126 134

Fig. 1.—Frequency chart showing the range of serum inorganic sulphate in normal adults and in patients with essential hypertension.

Undetermined organic acid was estimated by subtracting the total of the determined anions from the total base. In normal serum, these undetermined acids ranged from 3 to 6 milliequivalents per liter.

RESULTS

In Tables I, II and III are presented the results of the chemical studies made on all sixty patients. The data from twenty-seven patients with hypertension with comparatively little renal involvement are shown in Table I. In Table II a miscellaneous group of cases of Bright's disease are presented. Table III includes the electrolyte balance studies made on those patients who were in or near uremia. With the exception of the last two cases, in which cardiac failure was the most prominent finding, the cases in Table III are given in the apparent order of severity of uremic symptoms.

TABLE I

THE RELATIONSHIP BETWEEN SERUM INORGANIC SULPHATE, BLOOD UREA, AND UREA CLEARANCE IN CASES OF ESSENTIAL HYPERTENSION

CASE	SEX AND AGE	BLOOD UREA MG. N PER 100 C.C	SERUM SULPHATE MG. S PER 100 C.C.	UREA CLEARANCE PER CENT OF NORMAL
1	F 24	9.7	0.85	67
2	M 21	19.8	0.92	42
4	F 54	17.4	0.89	58
5	M 28	17.3	0.78	111
6	F 42	12.9	0.88	58
7	M 52	19.4	0.93	96
11	F 46	24.1	0.71	76
12	F 48	15.4	0.81	42
17	M 37	15.1	1.26	71
19	M 55	15.1	0.72	57
20	F 38	19.2	0.68	57
22	F 38	9.7	0.45	74
23	F 36	9.0	0.63	35
25	M 50	23.3	1.06	69
26	M 35	14.4	1.02	74
28	F 27	9.7	0.73	51
29	F 26	10.7	0.55	42
30	M 55	13.0	0.98	80
36	M 23	21.6	0.91	68
39	M 67	12.5	0.39	48
40	M 54	19.0	0.80	34
44	M 56	16.0	0.43	98
45	F 42	13.6	0.60	53
46	M 45	30.4	0.68	52
47	F 62	11.8	0.94	45
50	F 40	22.7	0.73	35
55	F 40	11.0	0.67	44

TABLE II

THE RELATIONSHIP BETWEEN SERUM SULPHATE, BLOOD UREA AND UREA CLEARANCE IN MISCELLANEOUS TYPES OF BRIGHT'S DISEASE

CASE	SEX AND AGE	BLOOD UREA MG. N PER 100 C.C.	SERUM SULPHATE MG. S PER 100 C.C.	UREA CLEARANCE PER CENT OF NORMAL	DIAGNOSIS
3	M 42	37.2	2.87	13	Malignant nephrosclerosis
8	M 53	176.1	6.60	4	Malignant nephrosclerosis
10	F 36	26.4	0.77	25	Chronic glomerulonephritis
18	M 40	33.2	0.73	30	Chronic glomerulonephritis
21	M 74	22.5	0.49	24	Arteriosclerosis
31	M 55	19.0	1.42	31	Cardiac decompensation
32	M 38	41.9	1.75	20	Cardiac decompensation
33	M 40	39.6	2.44	27	Cardiac decompensation
37	M 27	22.5	1.17	60	Amyloid nephrosis
38	M 55	169.0	7.90	–	Nephrosclerosis; coma
41	M 43	94.0	3.39	5	Malignant nephrosclerosis
43	M 48	35.4	0.89	43	Cardiac decompensation
49	M 21	25.4	1.09	50	Chronic glomerulonephritis with edema
51	M 31	36.2	1.50	16	Chronic glomerulonephritis with edema
60	M 34	44.0	2.70	22	Chronic glomerulonephritis with edema

TABLE III

CHEMICAL STUDIES OF THE SERUM FROM PATIENTS WITH TERMINAL BRIGHT'S DISEASE

CASE	SEX AND AGE	BLOOD UREA	UREA CLEARANCE	SERUM SULPHATE	TOTAL BASE	CHLORIDE	HCO_3	PROTEIN (B PR)	PHOSPHATE	UNDETERMINED ACID	SULPHATE	DIAGNOSIS
AUTHORS'	NORMALS	MG. N PER 100 C.C. 8-23	PER CENT OF NORMAL 75-120	MG. S PER 100 C.C. 0.4-1.1	M. EQ. PER LITER 153-158	M. EQ. PER LITER 100-106	M. EQ. PER LITER 25-28	M. EQ. PER LITER 15-17	M. EQ. PER LITER 2-3	M. EQ. PER LITER 3-6	M. EQ. PER LITER 0.3-0.7	
16	M 43	40.8	27	0.95	154.2	106.2	25.5	17.8	2.6	1.2	0.6	Nephrosclerosis, not in uremia
13	M 58	102.2	7	4.30	153.2	105.7	22.8	16.4	3.2	2.4	2.7	Malignant nephrosclerosis; beginning uremia
52	M 32	118.8	7	4.83	153.1	103.0	24.4	14.3	4.1	4.3	3.0	Chronic glomerulonephritis; beginning uremia
15	M 24	82.9	11	2.44	152.0	106.0	22.8	14.1	3.0	4.6	1.5	Chronic glomerulonephritis; beginning uremia
53	M 51	132.1	4	3.21	151.5	110.0	13.5	15.4	3.9	6.6	2.1	Malignant nephrosclerosis; beginning uremia
27	M 41	158.1	--	8.60	149.0	108.9	12.8	13.2	5.9	2.8	5.4	Malignant nephrosclerosis; uremia
48	F 42	60.0	6	1.62	155.0	110.0	25.0	15.1	2.8	1.1	1.0	Nephrolithiasis with hydronephrosis
54	F 43	115.1	--	3.00	138.6	102.2	8.9	15.9	4.7	5.0	1.9	Same patient in uremia 3 months later
34	M 42	162.5	4	6.23	146.7	99.2	17.9	15.0	5.6	5.1	3.9	Malignant nephrosclerosis; uremia
14	M 25	172.6	4	11.10	144.4	94.0	17.9	16.4	7.1	2.1	6.9	Chronic glomerulonephritis; uremia
56	M 17	260.1	--	12.74	145.0	100.8	9.0	11.6	9.3	6.3	8.0	Chronic glomerulonephritis; uremia
35	M 41	245.1	--	12.26	141.8	90.4	15.1	14.5	9.3	4.8	7.7	Chronic glomerulonephritis; uremia
58	F 46	157.1	--	7.71	135.8	87.7	15.3	12.8	7.1	8.1	4.8	Nephrosclerosis; uremic coma
24	M 38	217.0	--	7.49	146.1	95.5	7.7	19.7	7.5	5.0	4.7	Malignant nephrosclerosis; uremic coma
42	M 39	186.0	--	8.34	142.0	93.2	15.3	16.4	7.3	4.6	5.2	Malignant nephrosclerosis; uremic coma
59	F 31	186.2	--	8.82	132.6	80.3	13.7	16.0	9.1	8.0	5.5	Malignant nephrosclerosis; uremic coma
9	M 55	111.0	--	2.26	156.0	112.1	20.0	16.7	2.2	3.6	1.4	Cardiac decompensation; coma
57	M 41	70.0	--	0.66	149.2	113.5	19.1	9.5	2.4	4.3	0.4	Cardiac decompensation; coma

The patients with essential hypertension showed in almost all cases normal serum sulphate as well as urea. This was true even in those cases in which urea clearance was already below the minimal normal value of 75 per cent. Even those patients who already showed elevations of blood urea gave most often normal sulphate values. In the miscellaneous group of patients, it was found too that serum sulphate was usually within normal limits, and that not until urea nitrogen was distinctly elevated above normal was there a consistent rise in sulphate. There are several exceptions (Cases 17, 31, 37). In these, sulphate was elevated even though blood urea nitrogen was still below the maximal normal of 23 mg. per 100 c.c. Two of these cases (31 and 37) had the complicating factor of edema.

These findings are perhaps better visualized when expressed graphically. In Fig. 1, the distribution of serum sulphate in twenty-seven cases of hyper-

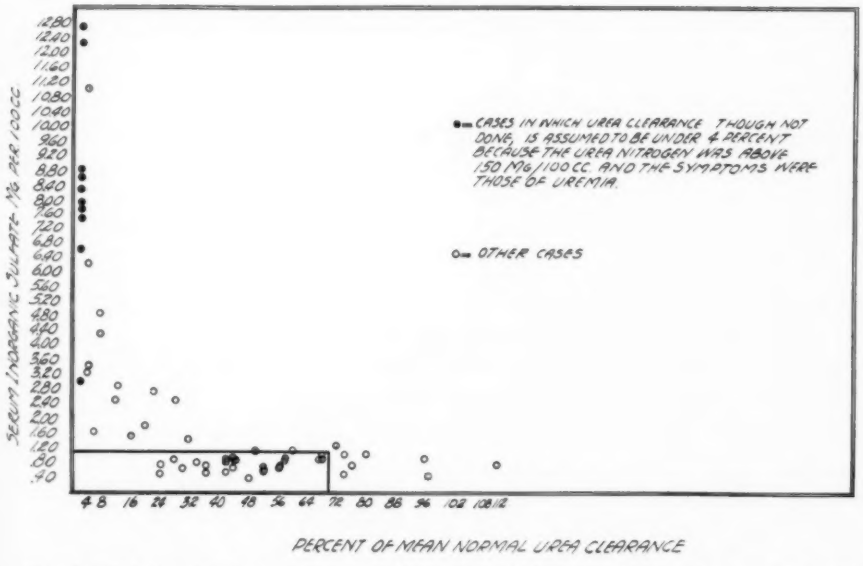

Fig. 2.—Relationship between serum inorganic sulphate and urea clearance in patients with hypertension or Bright's disease. The points enclosed in the rectangle represent sulphate values found within normal limits when the urea clearance already showed renal function less than the normal minimum.

tension is compared with that of the forty-two normal persons studied by Hoffman and Cardon.[4] The distribution, with the exception of one value, is obviously similar.

In Fig. 2, serum sulphate is plotted against the urea clearance in all the cases studied. In some of the uremic patients, it was impossible to determine urea clearances. In such patients, with blood urea nitrogen of 150 mg. per 100 c.c. or higher, it has been found by Van Slyke and others[13] and by other investigators including ourselves that the urea clearance was most often below 4 per cent. Such an assumption has therefore been made in these cases. The plotted values in this chart have a logarithmic distribution, sulphate tending to remain constant until urea clearance had dropped to low values, then rising rapidly as urea clearance sinks still further. It can be seen that

twenty-four cases still showed normal sulphate values when the urea clearance had already dropped below the minimal normal.

The logarithmic distribution of values in Fig. 2 is similar to that found by Van Slyke and his coworkers[13] when they plotted blood urea nitrogen against urea clearance. Such a similarity suggests a correlation between the levels of serum sulphate and urea. Fig. 3 illustrates this correlation more directly. Serum sulphate is seen to remain around normal until urea N rises to about 35 mg. per 100 c.c. Then the two values rise together, so that in uremia both are from 10 to 15 times as high as normal.

In Table III, sulphate, like other serum anions, is expressed in terms of milliequivalents of base neutralized. In this way, the rôle of sulphate in producing a lowering of the alkali reserve (as expressed by HCO_3) can be estimated. In uremia, sulphate is seen to have risen to as high as 8 milliequivalents per liter, which per se would contribute considerably to the

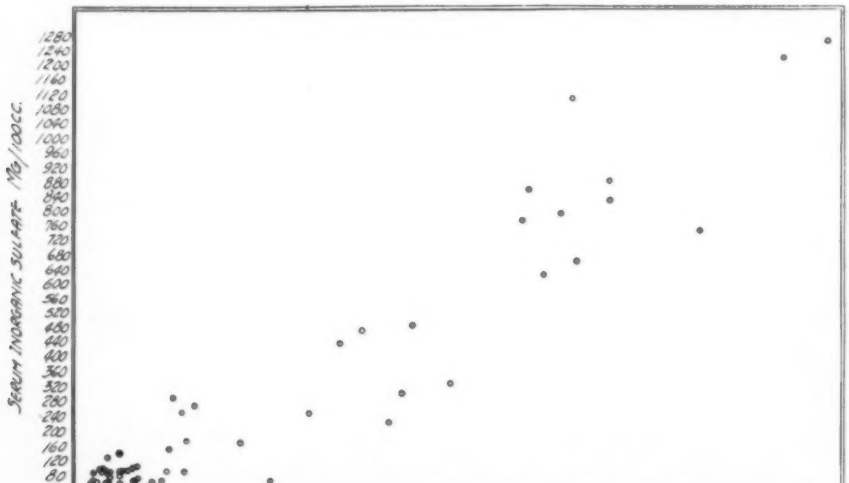

Fig. 3.—Relationship between serum inorganic sulphate and blood urea nitrogen in patients with hypertension or Bright's disease.

production of acidosis. In all cases of markedly elevated sulphate, phosphate had similarly risen. The rise in the two anions accounts for most of the drop in bicarbonate. In a number of cases, however, the lowered bicarbonate was found partly due to a lowering of total base without an equivalently great reduction of chloride. In fact, in several cases (53, 27, 57) serum total base was low while chloride was even higher than normal. Whether this condition is a genuine phase of uremia or whether it is due to the particular clinical management of these patients could not be determined. In severe uremia, particularly after gastrointestinal symptoms had become prominent, serum total base and chloride were both correspondingly low, and the diminished alkali reserve could be entirely accounted for by the rise in sulphate and phosphate. It should be further pointed out that serum sulphate was highest when serum total base was very low.

COMMENT

Sulphate, like urea, is a metabolic waste product eliminated from the body chiefly by way of the kidneys. One should therefore expect to find the level of serum sulphate related to the degree of renal efficiency. The results obtained here add confirmation to the already ample evidence of this relationship as reported by Denis,[1] Meyer-Bisch,[14] Loeb and Benedict,[15] Cuthbertson and Tompsett,[16] Wakefield and others,[2] and Macy.[17] When the renal function is greatly impaired, sulphate is retained in the blood.

On the other hand, it is hardly to be expected that sulphate retention should occur when the compensating power of the kidneys and circulation is still good, as expressed by normal concentration tests, normal phenolsulphonephthalein excretion, or by normal urea clearance. The present investigation could find no confirmation of the claims of Wakefield and his coworkers of such an early rise in sulphate. On the contrary, many of the hypertension cases reported here showed a lowering of urea clearance to about 50 per cent of the mean normal, without any other indication of renal involvement. In all these cases, with one exception, sulphate was within normal limits. In several cases, too, when urea was already distinctly elevated, serum sulphate was still within normal limits. It seems, therefore, that serum sulphate concentration cannot be used as a test for early renal inefficiency.

The roughly parallel rise of both sulphate and urea in advanced Bright's disease, as shown in Fig. 3, has already been mentioned. Neither Denis nor Wakefield could recognize any distinct correlation between urea and sulphate retentions. But Cuthbertson and Tompsett[16] and Loeb and Benedict[15] did affirm such a relationship. Fig. 3 undoubtedly shows a great deal of scattering, and the number of cases is probably too small to make a quantitative estimation of correlation. But it is quite apparent from this chart that when renal efficiency is markedly impaired, serum sulphate rises with a rapidity similar to that of urea.

The cause of the acidosis in uremia has been given considerable attention by investigators of Bright's disease. This question is thoroughly discussed by Peters and Van Slyke[12] and by Peters.[18] The primary disturbance is probably the great loss of fixed base in the urine produced by diuresis and by the inability of the damaged kidneys to convert a sufficient amount of urea into ammonia for the purpose of neutralizing excreted acids. This loss of base is reflected internally first as dehydration and second as a lowering of the serum total base concentration. All the uremic patients in the present group showed this phenomenon. However, only in the absence of a corresponding loss of chloride does this loss of base produce a considerable lowering of the alkali reserve. Cases 15, 27, 53, 54, 56, and 57 illustrate the diminution of alkali reserve caused in part by the unbalanced loss of total base and chloride. But in other cases, it is chiefly the retention of sulphate and phosphate that is responsible for the lowered bicarbonate. The relatively undisturbed level of undetermined acids shown in Table III adds confirmation

to the belief that in uremia there is little retention of unknown organic acids, unless a superimposed cardiac failure has allowed an accumulation of lactic acid, as pointed out by Meakins and Long.[19]

These findings are essentially the same as those recently reported by Atchley and Benedict,[20] by Briggs,[21] and by Greene, Wakefield, Power, and Keith,[22] who used other methods for the determination of serum total base and serum inorganic sulphate. However, the extent of the sulphate retention reported here is much less than that found by these authors. The maximum serum sulphate reported by Atchley and Benedict was 19.5 m.eq. per liter, by Briggs, 9.2 m.eq. per liter, by Greene, Wakefield, Power, and Keith, 16.2 m.eq. per liter, whereas the maximum found by the present sulphate method was 8.0 m.eq. per liter. In the paper by Wakefield, Power, and Keith,[2] where no electrolyte studies were made, values of 20 m.eq. or more per liter are suggested. Such high values are improbable, as Peters[18] has pointed out. The rise in serum sulphate, then, is an important factor in the production of nephritic acidosis, but the rôle it plays is probably less significant than that assigned to it by recent investigators.

In spite of the probability that the values for serum sulphate obtained by Wakefield and his coworkers are too high, one cannot entirely dismiss their findings of higher than normal sulphate in many early cases of Bright's disease. Many of their cases which showed this early rise had the complicating factor of edema. Several of our cases, too, of edema of either nephrotic or cardiac type showed an unexpected elevation of serum sulphate (Cases 31, 33, 37, and 60). In such cases of edema, chloride is at times also found higher than normal, even when serum total base is normal or lower than normal.[23] These findings suggest that factors other than renal efficiency may be concerned with the excretion of sulphate. Such a possibility is further enhanced by the work of Macy,[17] who showed that urinary sulphate excretion in normal persons varies markedly throughout the day even though serum sulphate concentration changes comparatively little. Denis and others,[24] too, found that diuretic measures can produce a rapid diminution in serum sulphate. The amount of sulphate excreted, even with normal kidneys, may depend upon the amount of base simultaneously available for excretion. If this is so, one can explain not only the high sulphate in cases of edema but also the apparent lack of correlation between urea and sulphate retentions in some advanced cases of Bright's disease. In order to throw more light on this subject, the authors are at present studying sulphate clearances under varying conditions of base excretion.

SUMMARY

A new method for the determination of serum inorganic sulphate has been developed, which avoids the errors of previous methods and which gives equally consistent results both with normal serum and that from pathologic cases. The average concentration of serum sulphate in forty-two normal persons was 0.78 mg. S per 100 c.c. with a standard deviation of ± 0.16 mg.

Sixty patients with hypertension or Bright's disease were studied for the relationship of serum sulphate to blood urea and urea clearance.

In twenty-seven persons with essential hypertension, serum sulphate had a distribution similar to that of normal persons.

There was no evidence that a rise of serum sulphate was often the first indication of renal inefficiency. On the contrary, in twenty-four out of sixty persons studied, serum sulphate was still normal when urea clearance was already below the minimal normal level. In some of these patients, blood urea was already distinctly elevated. Serum sulphate determinations are therefore of little help in determining early renal inefficiency.

In patients with terminal Bright's disease, serum sulphate rose roughly parallel to blood urea, and reached values of 12 mg. S per 100 c.c. or 8 milliequivalents per liter.

An analysis of the electrolyte balance in eighteen cases of uremia showed that the rise of serum sulphate, like that of phosphate, played a considerable rôle in the production of uremic acidosis.

These results are discussed and the suggestion is made that factors other than the degree of renal excretory efficiency may also be concerned with the level of serum inorganic sulphate.

The patients studied in this investigation were from the dispensary of The Chicago Medical School and from the wards of the Cook County Hospital. The authors wish to express their appreciation of the cooperation of the staff of the Cook County Hospital.

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WATER RETENTION IN OBESITY AS DETERMINED BY THE VOLHARD DILUTION AND McCLURE-ALDRICH TESTS*

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THERE is a growing belief in this country that obesity is purely alimentary in nature. The champion of this school of thought is Newburgh and his associates.[1] In the continental clinics the problem of obesity is looked upon as of dual character—exogenous and endogenous or constitutional. In practice it is quite difficult to know where exogenous obesity ends and endogenous begins, and in treating the two types of cases very little difference is found in their reaction to the dietary regimen. The purely mechanistic conception of Newburgh, however, does not fully account for certain well-known clinical observations, such as the accumulations of fat limited to certain localized areas of the body in some types of obesity; for example, in the eunuchoid type of individual, fat tends to accumulate in the gluteal region, hips, breasts, and mons pubis. Such an individual may be made at times to lose weight on a restricted diet, yet in the locations where fat accumulates most, the fat is likely to remain. The girdle obesity of pituitary disease and of Dercum's disease, in which fat accumulates in isolated masses in places subject to pressure, while the face and extremities remain uninvolved, would strongly suggest that besides energy intake, other factors, particularly endocrine, are involved.[2] This contention is borne out by the experience of Skoptzi, who developed suprapubic and trochanteric pads of fat after castration, as well as by the frequency with which obesity follows childbirth and menopause. The animal breeder has known for a long time that by means of castration he may readily fatten his stock. The rôle that the endocrine glands play in regulating energy balance and tissue activity is well recognized, but the manner in which a dysfunction of one or another of these glands affects this regulation is not as yet clear. It is interesting

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in this connection to call attention to the observations of Wade H. Brown in regard to obesity in rabbits, which he believes to be of hereditary and endocrine origin rather than dietary in character.[3]

The origin of obesity will be better understood when a solution is offered to explain how normal weight is maintained, and to explain further why the obese do not continue to gain in weight indefinitely but reach a level of stability for long periods of time. It was Van Noorden's belief that economies in the total metabolism of the obese were conditioned by a hypothyroid state.[4] This he assumed to be evidenced by a low basal metabolic rate. As a matter of fact, the basal metabolic rate is so irregular and inconstant in obesity as to be of no great moment in elucidating the pathogenesis of the disease. In fifty-two cases of obesity in which the patients were under observation at the Ductless Gland Clinic of Temple University Medical School from April, 1931 to April, 1933 only seventeen had a perceptibly low basal metabolic rate. Three patients had unusually high metabolic rates. Two of these, with obesity of cerebral origin, had rates of plus 30 per cent, and plus 37 per cent, respectively, while the third patient, who later developed typical thyrotoxicosis, had a rate of plus 47 per cent. Twenty-nine patients showed basal metabolic rates within the range of normality (see Table I). The extremely low basal rate that is occasionally observed in obesity is not characteristic of the disease. It is well known that a decreased basal metabolism is found in undernutrition, in pituitary emaciation (Simmond's disease), and in advanced Addison's disease. Obesity is not necessarily a symptom of hypothyroidism; at the present time we have three patients who are underweight rather than overweight, and who present otherwise typical symptoms of hypothyroidism with metabolic rates varying between minus 18 per cent and minus 22 per cent.

TABLE I

BASAL METABOLIC RATE IN OBESITY. TOTAL NUMBER OF CASES, 52

	NUMBER OF CASES
Below 20%	8
−11% to −19%	9
− 1% to −10%	11
Normal	5
+1% to +10%	13
Over +10%	6

Strang and Evans[5] have recently pointed out that when basal metabolic rates are estimated on the basis of active tissue mass rather than on surface area, it will be found that metabolism in obesity proceeds on a high rather than on a low level.

Specific Dynamic Action of Food.—That there are isolated obese patients who are refractory to the specific dynamic action of food is well attested to by the work of Rolly,[6] Plaut[7] and others. However, other investigators found normal or higher figures than for normal control persons.

One of us[8] has studied the specific dynamic action of food in a group of obese patients after a mixed meal and in another group after a protein meal

(see Tables II and III). In the first series the readings were taken at the end of forty-minutes and one and one-half hours after the mixed meal. Subsequently the method was altered and the reading was taken at the end of the first and second hours after ingestion of a protein meal consisting of 100 gm. of broiled chopped beef and 100 c.c. of water. The results of these observations are presented in Figs. 1 and 2. After the mixed meal only in one patient was there observed a complete absence of specific dynamic action of food. This patient was suffering from dystrophia adiposogenitalis. In another patient with a thyroovarian obesity the specific dynamic action of food was considerably increased over the normal. It is rather interesting to note that this patient was intolerant to thyroid therapy. One-half grain of desiccated thyroid gland three times a day for ten days produced marked symptoms of thyrotoxicosis. The stimulating effect of a protein meal was

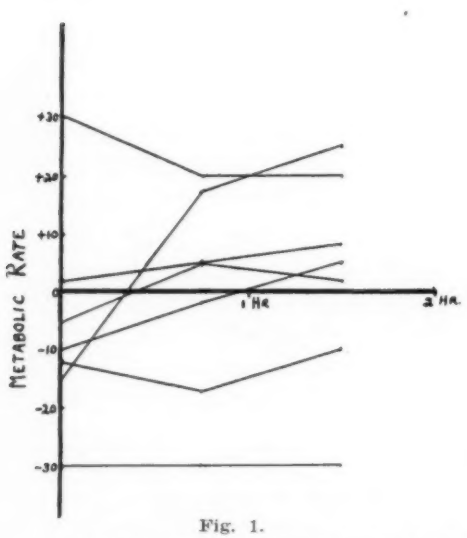

Fig. 1.

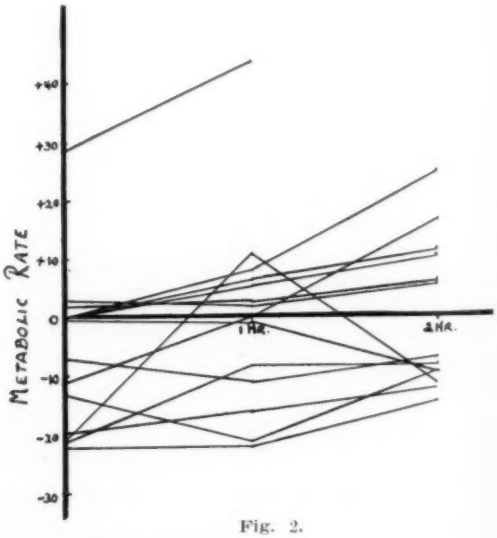

Fig. 2.

Fig. 1.—Effect of ingestion of a mixed meal on heat production.
Fig. 2.—Effect of ingestion of a protein meal on heat production.

computed in fourteen obese patients; the results were essentially the same as obtains in normal persons with the exception of three patients. Two of these were of the dystrophia adiposogenitalis type and one of obesity incident to menopause. In these three patients the response to the protein meal was low (see Table III). If one bears in mind the great variations in specific dynamic response of normal individuals, it is apparent that no definite opinion in general can be formed as to the rôle this factor plays in the pathogenesis of obesity.

Bernhardt[9] claims to have found long periods of depressed metabolism (negative phases) in the obese after light work and after a meal. Later investigators failed to confirm these findings.[10]

Water Retention.—In planning a therapeutic program for an obese person the problem of water metabolism should be taken into account. How great

a loss of body weight may be due to loss of water may be appreciated from the work of Gamble, Ross and Tisdall.[11] They computed the water weight lost during fasting in terms of fixed bases and thus were able to determine

TABLE II

EFFECT OF INGESTION OF A MIXED MEAL ON HEAT PRODUCTION

NAME	AGE YEARS	WEIGHT POUNDS	HEIGHT INCHES	BASAL RATE	METABOLIC RATE		PER CENT DIFFERENCE FROM BASAL OF PATIENT	
					45 MIN. AFTER MEAL	90 MIN. AFTER MEAL	45 MIN. AFTER MEAL	90 MIN. AFTER MEAL
J. K.	13	199	60	−30	−30	−30	0	0
R. B.	17	159	63	− 9	− 2	+ 5	7 (+)	14 (+)
T. B.	38	290	67	+30	+20	+20	8 (−)	8 (−)
N. S.	38	155	58	−12	−17	−10	6 (−)	3 (+)
C. W.	38	237	70	+ 2	+ 5	+ 8	3 (+)	7 (+)
M. H.	41	204	61	−15	+17	+25	38 (+)	48 (+)
L. G.	42	332	64	− 5	+ 5	+ 2	10 (+)	7 (+)

TABLE III

EFFECT OF INGESTION OF A PROTEIN MEAL ON HEAT PRODUCTION

AGE	SEX	WEIGHT POUNDS	HEIGHT INCHES	BASAL RATE	METABOLIC RATE		PER CENT DIFFERENCE FROM BASAL OF PATIENT	
					1 HOUR AFTER MEAL	2 HOURS AFTER MEAL	1 HOUR AFTER MEAL	2 HOURS AFTER MEAL
57	F	236	64	−21	− 8	− 8	13 (+)	13 (+)
29	F	259	66	0	+ 7	+12	7 (+)	12 (+)
41	F	202	61	−21	+11	−11	32 (+)	10 (+)
15	M	234	67	−11	0	+17	11 (+)	28 (+)
13	M	151	65	+ 3	+ 2	+ 6	1 (−)	3 (+)
12	F	178	60	−22	−22	−14	0	8 (+)
21	F	227	65	−13	−21	− 9	8 (−)	4 (+)
44	F	202	62	0	0	− 9	0	9 (−)
45	M	270	71	0	+ 8	+26	8 (+)	26 (+)
32	F	269	64	0	+ 7	+11	7 (+)	11 (+)
15	F	157	63	−20	−16	−12	4 (+)	8 (+)
20	F	160	61	− 7	−11	− 7	4 (−)	0
23	M	166	61	+29	+44		15 (+)	
55	F	165	61	+ 2	+ 2	+ 5	0	3 (+)

the amount of water lost from cellular content and intracellular storage. Results in one of their cases, A. G., an epileptic child who fasted for fifteen days, showed that 62 per cent of the loss of body weight was due to loss of water. The figures follow:

	c.c.	
Intracellular water loss:		
Due to destruction of protoplasm	1,620	
Due to reduction of cell volume	470	
	2,090	
Extracellular water loss	320	
Total loss of body water	2,410	
Loss of body weight	3,920	gm.
Body weight loss due to water	62	per cent

In the study of water metabolism there are to be considered four sources of water to the organism and three sources of water elimination. The factors involved in water balance as given by Newburgh and his associates are:[12]

Water Exchange:

AVAILABLE WATER	GRAMS	WATER GIVEN OFF	GRAMS
A. Water drunk	----------	E. Water of urine	----------
B. Water of food	----------	F. Water of stool	----------
C. Water of oxidation	----------	G. Insensible water	----------
D. Preformed water	----------		

Clinical studies of water balance by computing only fluids ingested and fluids excreted in urine and stool do not represent completely the true conditions of water exchange. Besides the water a person drinks as such and the water contained in food (meats run over 50 per cent water, vegetables over 75 per cent water, etc.), water is formed from oxidation of organic hydrogen of food. Another source of available water is from oxidation of protoplasmic hydrogen during the breaking down of body material in the process of combustion, yielding the so-called preformed water. The total proportion of water formed by oxidation of food is in the neighborhood of 300 gm. for a person at light activity. The quantity of preformed water in the body has a more or less fixed relation to living tissue. With deposition of new tissue there is likewise a retention of a corresponding amount of water. Preformed water under normal conditions is too negligible in amount to be of clinical importance, but in starvation the amount may reach large proportions. In practice one sees patients who are on a strict diet far below energy liberated, and yet they fail to lose weight; the probable explanation of this failure to lose weight is the abnormal retention by the tissues of preformed water. It appears then that patients on subcaloric diets, although actually ingesting less fluids than on higher diets, derive significant amounts of water from preformed sources.

Water lost from the body is represented by urine, stool, and water evaporated from the skin and lungs—insensible loss. Urine usually is regarded as the major source of water loss from the body. However, it should be borne in mind that the insensible loss of water is continuous and under unusual conditions may exceed the amount lost through urine. Newburgh and his associates[13] estimated the daily insensible loss of water in persons at normal activity to average from 1,000 to 1,500 c.c. They also furnished an accurate account of water exchange in connection with the study of the total metabolism in obesity. They found that departures from predicted losses of weight were always accounted for by storage or loss of water.[14] Van Noorden in 1910[15] has already indicated the importance of water and salt restriction in treatment of obesity.

Oertel[16] showed that the quantity of water that entered the organism affects the accumulation and consumption of fat. It was also observed that when the loss of water was greater than the intake the fat accumulated in the body decreased. Zondek[17] described patients who retain water as of the salt water obesity type. Bauer[18] speaks of them as cases of hydrolipomatosis.

In 1931 one of us[19] called attention to the importance of water storage as a contributing factor in obesity and reported satisfactory results from the

intravenous use of salyrgan in a group of patients who failed to lose weight on a strict submaintenance diet, the stationary level of weight being doubtless due to hidden water retention. Rowntree and Brunsting[20] later reported two cases of obesity characterized by striking retention of water and in whom weight loss was induced by salyrgan.

To determine latent water retention in obesity we have been using the Volhard dilution test and the McClure-Aldrich skin test.

Volhard Dilution Test.—Method: The patient is given 1,500 c.c. or 1,000 c.c. of tap water on an empty stomach and the urine is collected at hourly intervals for the next four hours. During this test the patient is permitted to be up and around and to carry on his usual duties. The amount collected for four hours is measured, the specific gravity taken, and routine urinalysis is made on the mixed specimen. Patients who show cardiovascular disturbances or whose urine shows some deviation from the normal are eliminated as unfit for this test. According to Pratt,[21] a normal four-hour output by the Volhard test is equal to or greater than the intake. A more recent study by Bartels and Blum[22] seems to indicate that an output slightly below the intake may be regarded as normal, and after ingestion of 1,500 c.c., the average output of their control subjects was 1,334 c.c. Accordingly, we have accepted as abnormal only those results which show a negative balance of more than 200 c.c.

Results: Thirty-six patients from the Ductless Gland Clinic, Temple University Hospital, were studied. They were all more than 10 per cent overweight as compared with accepted normal weights. Of this group seven patients or 19 per cent had a greater output of urine than intake of water. Ten patients or 28 per cent had an output within the range of normality. Nineteen patients or 53 per cent had an output definitely less than the intake of fluid, averaging a negative balance of 515 c.c. in four hours. These figures, as given in Table IV, compare favorably with a previous study on dilution tests in obesity published by one of us[23] in 1932. This study preceded that of Malamud[27] to whom Bartels and Blum in their review of the subject refer as the first to describe studies of this nature.

It would appear that more than half of the obese patients in this study showed a disturbance in the water balance as evidenced by the Volhard dilution test. In the thirty-two subjects more than 10 per cent overweight studied by Bartels and Blum, severe water retention (output less than 1,000 c.c. after 1,500 c.c. intake) occurred in 28 per cent; the number of their patients who had a negative balance of 200 c.c. or more was twenty, or 62 per cent of the group. This latter figure coincides more closely with our own, and in our opinion represents a truer approximation of the incidence of water retention in obesity. Analysis of our data, as well as that of Bartels and Blum (see Tables V and VI), leads us to disagree with their conclusion that "obese subjects do not retain fluids to a great degree, as in only 7 per cent was the output less than 1,000 c.c." Careful study of their data shows that the 7 per cent applies to patients from 0 to 10 per cent overweight, and not to patients more than 10 per cent overweight into which class the obese individuals would fall. In comparing Bartels' and Blum's cases with our

own, it is to be noted that only five of their group were thirty or more pounds overweight, while sixteen patients of our group with heights from 60 to 65 inches were well over 200 pounds in weight. We must conclude, therefore, that our patients as a group represent pathologic obesity more so than do those of Bartels and Blum.

TABLE IV

VOLHARD DILUTION TESTS IN OBESE PATIENTS

NAME	SEX	AGE	WEIGHT POUNDS	HEIGHT INCHES	INTAKE C.C.	OUTPUT C.C.
M. S.	F	29	248	60	1,500	900
M. Z.	F	50	201	60	1,500	1,400
M. W.	F	48	276	64	1,500	750
M. Z.	F	13	196	63	1,250	1,100
L. R.	M	46	233	74	1,500	1,000
A. B.	F	40	275	62	1,000	1,000
T. B.	M	38	295	67	1,500	850
M. M.	F	42	202	64	1,000	625
E. L.	F	10	96	53½	750*	875
E. L.	F	39	202	63½	1,000	700
G. T.	F	63	184	64	1,500	1,045
L. C.	F	49	179	61	1,500	1,684
N. M.	F	50	192	62	1,500	687
E. K.	F	21	344	63½	1,500	890
M. S.	F	15	178	69	1,000	1,000
L. G.	F	26	210½	64	1,500	1,290
C. W.	F	63	168½	61½	1,000	1,000
H. A.	F	64	180½	59½	1,000	720
L. B.	F	35	210	66½	1,000	500
D. C.	F	32	270	64½	1,500	1,700
G. H.	F	33	170	63	1,000	500
M. D.	F	26	148	61¼	1,000	1,200
R. Z.	F	23	140	59	1,000	1,000
M. J.	F	37	183	63¼	1,000	1,250
J. R.	F	47	172	63	1,500	1,200
I. G.	M	42	332	62½	1,500	900
N. S.	F	38	155	67	1,500	1,700
N. T.	M	53	190	61	1,500	1,100
M. G.	F	23	200	63	1,500	900
M. H.	F	41	204	64	1,500	1,400
A. B.	F	43	244	64	1,500	1,500
A. H.	F	43	242	63½	1,500	1,350
J. W.	F	22	221	64	1,500	1,521
A. R.	F	42	156	62	1,500	500
B. M.	F	25	179	66	1,000	750
M. G.	F	30	164	63¾	1,000	685

*Could not drink full amount.

McClure-Aldrich Test.—Method: Two-tenths cubic centimeters of 0.85 per cent aqueous solution of sodium chloride is injected intracutaneously into the flexor surface of the forearm, inner surface of the thigh, the gluteal region, and the abdomen. A duplicate injection is made in areas that are comparatively free from fat deposits. The end-point of disappearance of the raised wheal is determined by palpation. When the elevation persists for sixty minutes, it is indicative that the tissues do not show increased avidity for water.

Results: In 15 patients of our series, both the McClure-Aldrich and the Volhard dilution tests were made. In 6 patients or 40 per cent of the group,

the McClure-Aldrich test showed rapid absorption in all areas tested, with definite retention by the Volhard test. In 6 patients, or 40 per cent, the results of the Volhard test were normal; in 3 of these the McClure-Aldrich test showed rapid absorption in all areas tested, and in the remaining 3 the McClure-Aldrich test yielded practically normal results. In 3 patients the Volhard test was normal, and the McClure-Aldrich test was positive only in one or two places tested (see Table VII).

TABLE V

(AFTER BARTELS AND BLUM)

RESULTS OF DILUTION TESTS ON THIRTY-TWO SUBJECTS WHO WERE MORE THAN 10 PER CENT OVERWEIGHT

OVER-WEIGHT POUNDS	CASE NO.	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
10	9	275	120	70	40	505
10	35	155	165	135	75	530
10	73	485	205	130	80	900
10	100	500	650	50	100	1,300
11	22	305	550	375	45	1,275
11	24	200	530	90	30	850
12	64	630	620	144	50	1,440
12	70	810	700	450	50	2,000
12	108	1,000	850	150	25	2,025
13	44	420	480	180	55	1,135
13	82	800	825	150	20	1,795
14	103	500	600	300	50	1,450
15	20	315	395	125	30	865
15	105	900	700	150	100	1,850
16	79	285	465	260	95	1,165
16	87	600	600	500	120	1,820
17	96	500	500	500	50	1,550
18	83	900	650	300	150	2,000
20	48	555	670	265	40	1,520
20	111	95	320	235	45	695
		35	200	475	350	1,060
		55	500	575	285	1,415
		195	600	350	70	1,215
21	107	550	650	400	110	1,710
22	3	350	875	210	52	1,487
22	11	165	500	145	35	845
22	60	155	375	70	50	650
24	14	550	360	175	60	1,145
29	34	250	435	490	120	1,285
29	58	350	570	290	110	1,320
31	113	395	380	455	320	1,550
		180	375	450	560	1,565
		270	470	470	235	1,445
38	109	210	580	460	35	1,285
		100	590	435	175	1,300
		170	725	430	75	1,400
		210	605	480	220	1,515
40	110	235	620	185	40	1,080
		175	580	355	90	1,200
		250	655	240	55	1,200
46	112	240	310	330	220	1,200
		365	410	300	200	1,275
		225	395	345	205	1,170
		270	230	250	210	1,070
75	15	170	280	140	115	705
		265	265	450	230	1,310

TABLE VI

(AFTER BARTELS AND BLUM)

PER CENT OF SUBJECTS IN EACH GROUP WITH OUTPUT OF MORE THAN 1,500 C.C. AND LESS THAN 1,000 C.C. OF URINE

MORE THAN 1,500 C.C.	PER CENT
0 to 10 per cent underweight	33
0 to 10 per cent overweight	40
More than 10 per cent underweight	28
More than 10 per cent overweight	34
LESS THAN 1,000 C.C.	
0 to 10 per cent underweight	23
0 to 10 per cent overweight	7
More than 10 per cent underweight	28
More than 10 per cent overweight	28

TABLE VII

MCCLURE-ALDRICH'S INTRADERMAL SALT SOLUTION TEST IN OBESITY

NAME	SEX	AGE	WEIGHT POUNDS	HEIGHT INCHES	ARM M.	ABDOMEN M.	THIGH M.	GLUTEAL M.	RESULTS OF VOLHARD TEST C.C.
M. S.	F	29	248	60	45	15	50	20	−600
M. Z.	F	50	200	60	55	20	45	50	−100
M. W.	F	48	276	64	--	15	25	18	−750
M. Z.	F	13	196	63	50	--	40	--	−150
L. R.	M	46	233	74	40	25	45	40	−500
A. B.	F	40	275	62	7	7	6	20	0
M. M.	F	42	202	64	13	2	3	29	−375
E. L.	F	10	96	53½	25	20	20	--	+100
E. L.	F	39	202	63½	20	19	25	--	−300
G. T.	F	63	184	64	3	8	8	20	−455
L. C.	F	49	179	61	8	57	57	60	+184
N. M.	F	50	192	62	5	23	29	--	−823
T. B.	M	38	291	67	7	--	10	--	−650
E. K.	F	21	344	63½	10	43	52	35	−610
M. S.	F	15	178	69	12	--	6	2	0

Patients who showed a positive McClure-Aldrich test, and normal Volhard test, were carefully studied for cardiac and renal disturbances but no cause could be assigned for the positive McClure-Aldrich test. The apparent discrepancy between the Volhard test, and the McClure-Aldrich test in these cases suggests that over certain fatty areas of the body there is a greater avidity for water than is manifested by the body as a whole. We realize that this series is too small to permit final judgment, but we feel that the McClure-Aldrich test cannot be relied upon to detect water retention in obesity for clinical purposes with the same degree of accuracy as the Volhard dilution test.

CONCLUSIONS

In obesity the law of conservation of energy is operative, i.e., the balance between energy output and energy intake is disturbed. From laboratory tests it cannot be deduced with certainty that the underlying factor of this disturbance is conditioned by an endocrine dysfunction. In a large majority

of obese patients, both the basal metabolic rate and the specific dynamic action of food are within normal limits. Clinically, however, many of these patients may present stigmas of single or more often of mixed endocrinopathy. An additional factor which appears to play an important rôle in obesity is a disturbance of water metabolism which may lead to hidden water retention not evidenced by pitting edema. In planning a therapeutic regime this factor should be taken into consideration. The Volhard dilution test may be used as a simple, clinical measure to detect obese patients who tend to retain water.

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EFFECTS OF HYPERPYREXIA ON THE HUMAN BLOOD COUNT, BLOOD CHEMISTRY AND URINE*

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ARTIFICIAL fever has now become a recognized adjunct in the treatment of many diseases, including syphilis, multiple sclerosis, acute and chronic arthritis, pelvic inflammatory disease, and asthma. The stimulus of popularity has caused the physiologic aspects of hyperthermia to be investigated carefully; however, contradictory findings have been reported, so that work of this kind is still of interest in obtaining knowledge of the body functions during fever.

Haldane and Priestley[1] in 1905 showed that hot baths produced a deficit of CO_2 in the blood by hyperventilation of the lungs. Barbour[2] demonstrated in 1920 that hot baths increased the fluid content of the blood, a fact that was confirmed by others.[3, 4, 5] Henderson, Prince and Haggard[6] had, in 1918, showed that hyperventilation without fever would produce a slight thinning of the hemoglobin corresponding to the degree of apnea, and this fluid balance of the blood has been developed on a chemical basis.

Following the initial dilution the blood then becomes concentrated during continued fever by loss of fluid through the lungs, sweat glands, and kidneys.[7, 8]

Different reports are to be found concerning the blood sugar values after hyperpyrexia. It is reported[7, 9] that increase in body temperature has a stimulating effect on metabolism as shown by a rise in the respiratory quotient and fall in the blood sugar level. However, Lépine[10] found the blood sugar little altered in fever, and where increased he believed it was due to irritating effects of fever toxins on the fourth ventricle. Freund and Marchand[11] declared that an environment sufficient to raise a rabbit's own temperature will also give hyperglycemia. Dennie,[12] using hot baths, has shown an increase in the blood sugar reading.

Uric acid and nonprotein nitrogen increase and the CO_2 capacity decreases.[8, 12] According to Neymann and Osborne[8] the blood chlorides are slightly increased, but Osborne[13] in a later report with Markson states that either no change or a slight decrease takes place, the amount of chloride lost from the body during an average fever treatment being from 18 to 24 gm.

The effect of hyperpyrexia on the blood count has likewise been carefully studied. Bierman[14] observed an initial fall in white cells followed by an increase, due in part at least to stimulation of the hematopoietic system as evidenced by appearance of immature cells. He confirms the finding of

*From the Department of Internal Medicine, University of Kansas School of Medicine. Received for publication, June 3, 1935.

Hinsie and Carpenter[15] that the maximum increase occurs about the ninth hour, even after the temperature has dropped to normal. The hemoglobin and red count are increased mainly by dehydration, but in part to stimulation of the bone marrow.[16] The lymphocytic series decrease in accordance with the relative increase in granulocytes.[13]

With this rather conflicting literature we have made a study of some of the physiologic changes taking place during routine hyperpyrexator treatments, hoping that the findings would be of use in evaluating this form of therapy. Our report is based on the average findings in seventy-one treatments including ten patients. Most of these were ambulatory and were selected from the out-patient department. The cases included chronic arthritis, hypertension, pelvic abscess, central nervous system syphilis, gonorrheal urethritis, and multiple sclerosis.

Method.—Production of fever was accomplished by use of a standard hyperpyrexator cabinet, using resistance coils to furnish heat and employing no fans or circulating devices. It was therefore a production of fever by contact of heated air with the body surface. Urine was voided and blood obtained from the antecubital vein for blood count and blood chemistry before entering the cabinet and again after the patient's temperature had returned to normal. Water was given freely but no drugs or salt allowed. The oral temperature was recorded every ten minutes, which interfered in no way with the treatment. A few continuous records of axillary temperature were taken but were not very satisfactory. The average length of time at which the temperature was above normal was 3.7 hours. The average peak temperature was 103°. A trained nurse was in constant attendance and recorded the pulse and respiration with each temperature.

Results.—The urine in most cases showed a tendency to become alkaline, although this was but roughly estimated with litmus. There was always concentration of urine during treatment and some patients were unable to void any urine even after an interval of four hours from the previous voiding. Very little change in the sediment was observed. Occasionally where albumin appeared before the treatment, none was seen in the second specimen and in the same manner, hyaline casts showed a tendency to decrease during treatment. No sugar was observed in any of the specimens, either before or after the period of fever, even though in one patient blood sugar readings as high as 190 mg./100 c.c. were found following return of normal temperature.

Blood chemistry determinations were limited to sugar, nonprotein nitrogen, creatinine, and chlorides. The average readings are as follows:

	BEFORE TREATMENT	AFTER TREATMENT
Sugar	99.9 mg./100 c.c.	121.3 mg./100 c.c.
Nonprotein nitrogen	27.4 mg./100 c.c.	30.8 mg./100 c.c.
Creatinine	1.23 mg./100 c.c.	1.4 mg./100 c.c.
Chlorides	485.3 mg./100 c.c.	474.1 mg./100 c.c.

The blood counts included hemoglobin, red cell count, white cell count, polymorphs (filamented and nonfilamented), and lymphocytes. Other types of white cells were not included in computing the averages because of their

infrequent appearance. No reticulocyte counts were attempted. The average results of the blood counts were as follows:

	BEFORE TREATMENT	AFTER TREATMENT
Red cells	4,530,000/c.mm.	5,258,000/c.mm.
Hemoglobin	85.0%	92.4%
White cells	6,270/c.mm.	10,590/c.mm.
Neutrophile polymorphs	68.1%	81.6%
Filamented	50.2%	58.7%
Nonfilamented	17.9%	22.9%
Lymphocytes	31.9%	18.4%

Discussion.—Concentration of the blood during protracted fever may be expected, although Tenney[16] says that if fluids are given abundantly, the viscosity of the blood remains the same. The hemoglobin increased approximately 9 per cent, which we assume to be entirely due to concentration. Reticulocyte counts would be of value in determining whether stimulation of the red blood forming mechanism actually takes place. Creatinine increased 14 per cent and nonprotein nitrogen 13 per cent, a fact which may indicate some destruction of the body tissue, since, other factors being equal, increase due to concentration alone should approximate the findings of the hemoglobin. Perhaps the most striking finding is the rise in blood sugar. With the known increase in the respiratory quotient during fever, it might be assumed that the blood sugar level would be decreased. Actually we found the average increase to be over 20 per cent. Dennie,[12] who reports the same results, believes this is due to central stimulation through the autonomic system. This phase needs more investigation, especially where stimulation of the adrenals may be the responsible factors. This elevation of blood sugar is transient, and although we have had readings as high as 190 mg./100 c.c., no sugar spilled over into the urine. We substantiated the findings of Markson and Osborne[13] that the chlorides show a slight decrease. The average drop was 2 per cent.

The white cell count showed an increase of 68 per cent, thus allowing 9 per cent as that due to concentration of blood, we find 59 per cent increase which must be accounted for either by stimulation of the white blood forming organs or by a pouring out of these cells from storage depots within the body. We found the increase in nonfilamented cells to be 30 per cent, while myeloblasts, premyelocytes, and myelocytes were occasionally seen, thus indicating considerable stimulation to formation. The remaining 29 per cent of increase is accounted for by response of the reticuloendothelial system to the stimulation of heat as from infection, by pouring into the blood older forms of banded polymorphs. That the bone marrow responds more quickly than the lymphatic system is seen in the relative decrease of lymphocytes in the count following treatment. Stimulation of the lymphatic system occurs but more slowly than the white cell forming organs.

We believe that different methods may account for the different findings of investigators. Thus some of the work was done on animals and some on men. The fever in some cases was due to disease[3, 4] and in others the temperature was elevated by means of hot baths, short wave oscillations, electric heat, etc., permitting various factors to enter in. We feel that one of the

most important points in arriving at any conclusion is that a sufficiently large series of cases be studied to account for individual variation. Thus our averages include ten treatments of one patient who reacted very differently from the others, in that the blood sugar showed a decrease and the white cells were displaced by the lymphocytes. This despite the fact that her pelvic abscess was clinically cured!

SUMMARY AND CONCLUSIONS

1. Seventy-one treatments of ten patients by artificial fever have been studied for some of the commoner changes in the blood count, urine, and blood chemistry.

2. During treatment, the blood is first diluted, but later becomes concentrated.

3. The urine shows an increasing alkalinity and occasionally clearing of albuminuria.

4. The blood sugar level shows a marked rise, with no glycosuria when the level reaches to the accepted renal threshold or beyond.

5. Creatinine and nonprotein nitrogen show more of an increase than can be considered due to concentration.

6. Blood chlorides decrease approximately 2 per cent.

7. The white blood count is increased by fever treatments. This is first noticed in the granulocytes, and is due partly to production of immature forms and partly to mobilization of older types.

8. Any experimental work of this nature should be under standard conditions and should cover a sufficiently large number of cases to allow for individual variation.

We wish to express our thanks to Miss Esther Rodewald, Miss Muriel Smith, and Miss Dorothy Dixon, who performed the laboratory work, and Miss Blanche Marvin and Miss Ursula Brunner of the physiotherapy department for their careful supervision of the patients while under treatment.

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THE BLOOD SUGAR IN UNCOMPLICATED AND UNTREATED NEUROSYPHILIS*

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SYPHILIS when present in the human body can act as an irritant, can cripple, or destroy. When it attacks the central nervous system, it usually produces some form of neurosyphilis. In the floor of the third ventricle of the central nervous system there is an area which, when destroyed or irritated, will produce a hyperglycemia. Below it and attached to it is the pituitary gland. Irritative and destructive lesions of certain portions of this gland, especially the posterior and infundibular parts, will produce a hyperglycemia. A lesion of the pituitary may produce hyperglycemia indirectly by affecting the area in the floor of the third ventricle and/or the sympathetic-parasympathetic nervous conduction (and/or connection), or it may act through hormone influence on the thyroid, the suprarenals, or the pancreas.

There have been described lesions due to syphilis and which resulted in hyperglycemia in all of these areas. Hyperglycemia has also been described in abnormal emotional states. In neurosyphilis there is usually an associated abnormal emotional state. In view of these possibilities it was felt that a study of the fasting blood sugar in uncomplicated and untreated neurosyphilis was justified in as much as it might reveal some evidence of an abnormal sugar metabolism.

RESULTS

The study was composed of the fasting blood sugar of 207 cases of uncomplicated and untreated neurosyphilis. These cases subdivided themselves into 177 cases of general paresis, 22 cases of cerebrospinal syphilis with psychosis, and 12 cases of tabes without psychosis.

The method of estimation of the blood sugar was the Folin-Wu.[1]

Table I shows the salient facts concerning the blood sugar. The figures represent the number of milligrams of sugar in 100 c.c. of whole blood. Normal blood sugar is considered to be within the range of 80 to 120 mg.

*From the Psychiatric Clinic, Boston State Hospital.
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TABLE I

TYPE	NO. CASES	RANGE VALUES	MEAN	CASES WITHIN NORMAL	PER CENT	CASES BELOW NORMAL	PER CENT	CASES ABOVE NORMAL	PER CENT
Neurosyphilis	207	66-160	98.6	187	90.3	9	4.3	11	5.2
General paresis	177	66-154	97.8	160	90.2	8	4.5	9	5.0
Cerebrospinal syphilis with psychosis	22	66-104	92.9	20	91.8	2	9.1	0	0.0
Tabes without psychosis	12	76-122	100.8	9	74.9	1	8.3	2	16.6

DISCUSSION

The mean blood sugar values in syphilis of the central nervous system were all within normal limits for the types studied. It sometimes happens that although the mean in a group of estimations is within normal limits so many of the estimations are not within this range that the mean value is worthless and misleading. In the groups studied, however, this was not so. In the group neurosyphilis as a whole 90.3 per cent, in general paresis 90.2 per cent, cerebrospinal syphilis with psychosis 91.8 per cent, and in tabes without psychosis 74.9 per cent of the cases had fasting blood sugar values within normal limits. In those cases which had values below or above normal no pathologic reason for the abnormal fasting sugar could be discovered. It is very possible that these abnormal sugar estimations which constitute less than 10 per cent of the total group are due to some masked pathology of the regions suggested earlier, but lack of definite evidence is not conducive to definite statements in this respect. It is significant, however, that in over 90 per cent of the cases of uncomplicated and untreated neurosyphilis studied there was no evidence of any abnormality of sugar metabolism which could be demonstrated by a fasting blood sugar.

From this study there are two logical conclusions: (a) fasting blood sugar in neurosyphilis is usually within normal limits and (b) when an individual has neurosyphilis it is highly improbable that there will be a coincident abnormality in sugar metabolism which can be established by a fasting blood sugar.

SUMMARY

There has been presented a study of the fasting blood sugar in 207 cases of neurosyphilis. In 90.3 per cent of these cases this sugar was within normal limits.

REFERENCE

1. Folin, O., and Wu, H.: A Simplified and Improved Method for Determination of Blood Sugar, J. Biol. Chem. **41**: 367, 1920.

LABORATORY METHODS

A MOUSE PROTECTION TEST FOR STANDARDIZING ANTIMENINGOCOCCUS SERUMS*

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ASSISTED BY RENA SKLARSKY, B.A.

ABOUT six months ago we reported successful results in using a mouse protection test for standardizing antimeningococcus therapeutic serums.[1] The present paper is a further study of this test with especial reference to its practical application, and a comparison of the results obtained by the mouse protection test and by titrating the serums for agglutinins and precipitins.

The standardization of antimeningococcus therapeutic serums has been the subject of study for quite some time. Until recently the method generally employed was to determine the agglutinin, precipitin and, in some instances, the complement-fixing titer of the serums and consider them potent when they had a high titer of one or more of the above antibacterial antibodies.

The question of the rôle of agglutinins, precipitins, and complement-fixing antibodies in preventing or overcoming infection is still a matter of dispute. It is a well-known fact that in some antibacterial serums, such as antipneumococcus serums, the protective power of the serum does not run parallel with their antibacterial antibody content. The same may also be true of antimeningococcus serums. If a reliable test could be found by means of which the protective power of the serums against virulent cultures of meningococci could be determined, their therapeutic value could probably be more accurately standardized than by antibacterial content determination.

The main difficulty in carrying out protection tests against meningococci is that small laboratory animals are ordinarily not susceptible to meningococci unless large doses are given, and it is difficult to obtain a constant killing dose even with large doses. Zrodowski and Voronina[2] were able to infect rabbits by subarachnoid inoculation of meningococci. In our hands their method gave exceedingly variable results, and we, therefore, found it unsuited for routine tests. About two years ago Miller[3] reported successful infection of mice when recently isolated meningococcus cultures were suspended in a solution of mucin instead of saline and the suspension was injected intraperitoneally. By repeated passage he obtained cultures that killed mice in six to twenty-four hours.

We employed a slight modification of Miller's method to raise the virulence of some of our cultures as follows:

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Recently isolated cultures were maintained on freshly prepared infusion blood agar pH 7.2-7.4 and were transplanted twice a week. Before mice were inoculated the cultures were transplanted two or three times at twenty-four-hour intervals on large tubes of freshly prepared blood agar, selecting the tubes that contained considerable water of condensation, as such tubes usually gave a heavy mucoid growth within twenty-four hours. The twenty-four-hour growth from these tubes was washed off with a mucin solution that was prepared according to Miller's[3] method.* The mucin suspension of the culture was filtered through a thin layer of sterile cotton in order to eliminate clumps and was then standardized by opacity using McFarland's standard. It was further diluted with mucin to the desired concentration of organisms and 1 c.c. amounts of the various dilutions injected intraperitoneally into several mice on each dose. When the mice died, cultures were made from the heart's blood and mucoid colonies were selected from the growth for further passage.

We were able in this manner to obtain two virulent cultures of meningococcus Types I and III which produced fatal septicemia in mice when given in doses of 50 to 100 million cocci. Most of the mice died within eighteen to twenty-four hours and the cultures were recovered from the heart's blood. Some of the mice lingered for thirty to forty-eight hours, and in these mice the culture was seldom found in the heart's blood. Although the fatal dose of these strains is very much larger than that obtained by Miller, unselected white mice weighing 15 to 21 gm. were killed regularly and the cultures were recovered from the heart's blood in most instances.

Having obtained virulent cultures we then tested several preparations of antimeningococcus therapeutic serums for their power of preventing death in mice when a multiple of the killing dose of one of these cultures was given. The serums were diluted with mucin and the dilutions were made so that the desired dose was contained in 0.5 c.c. The culture was similarly diluted. One cubic centimeter syringes were used for injecting the test material and 0.5 c.c. of serum dilution was drawn up in the syringe followed by 0.5 c.c. of the culture, and this was immediately injected into mice.

It was found that the serums were able to protect the mice, in multiple proportions, while there was 80 per cent to 100 per cent mortality among the control mice that received the same amount of culture without the immune serum, and culture plus normal serum. The mice that received a sufficient amount of the immune serum remained well and were discarded; while those that received an insufficient amount of the immune serum, and the control mice, showed symptoms of illness after three to five hours and most of them died in eighteen to twenty-four hours. A small number lingered for twenty-seven to thirty hours and an occasional few recovered at the end of two days.

Our next problem was to determine the optimum method of administering the serum in testing its potency. Fifty mice were divided into 5 groups of 10 mice each. Group 1 was given the serum eighteen hours before the culture. Group 2 was given the serum and culture simultaneously. Groups 3, 4, and 5

*In a personal communication at a later date Dr. Miller advised the addition of 1 per cent glucose to the mucin which we have used in our later tests.

were given the serum one, three, and five hours after the culture. The results are given in Table I; the best results were obtained when the serum and culture were given simultaneously, although a considerable amount of protection was obtained three hours after the culture was administered and possibly larger

TABLE I*

MENINGOCOCCUS MOUSE PROTECTION TESTS. COMPARATIVE RESULTS OBTAINED BY DIFFERENT METHODS OF ADMINISTERING IMMUNE SERUM

IMMUNE SERUM PREP. 42	RESULTS					
	SERUM 0.005 C.C. + CULTURE 250 MIL. COCCI			SERUM 0.001 + CULTURE 250 MIL. COCCI		
ADMINISTERED	MICE INOC.	SURVIVED	PROTECTED PER CENT	MICE INOC.	SURVIVED	PROTECTED PER CENT
18 hr. before culture	5	2	40	5	0	0
Simultaneously	5	5	100	5	4	80
1 hr. after culture	5	5	100	5	1	20
3 hr. after culture	5	3	60	5	0	0
5 hr. after culture	5	1	20	5	0	0

*Controls—250 million cocci 5 mice none survived.

amounts of serum may have prevented death even at a much later period. Since the best results were obtained in this test when the serum and culture were given simultaneously, we selected this method for our subsequent tests of the protective power of various preparations of antimeningococcus therapeutic serums which were obtained from various sources including several of our own preparations.

We have tested so far 16 different preparations of antimeningococcus serums, 15 of these serums were tested with the culture 23047 which is a Type III, and 6 serums were tested with culture 24995 which is a Type I. Each serum was titrated for agglutinins and for precipitins as well as for its power to prevent death in mice when they were given 200 million cocci of the culture. Five mice were used for each dose of the serums as well as for the controls, where either culture plus normal serum or culture only was used. Unselected white mice were used in these tests, which varied in weight between 15 and 21 gm. Since mice of different weights show considerable difference in their susceptibility to meningococci we included in each group of 5, mice of the same weight in order to make the results of each test comparable. The results are given in Table II.

The results obtained in this experiment show that the agglutinin, and precipitin titers of the serums tested did not run parallel with their power to protect mice against a multiple of the minimum fatal dose of virulent meningococci. As shown in Table II, serums that had approximately equal agglutinin and precipitin titers differed considerably in their protective power (I No. 4 and 5). Also serums that differed widely in their agglutinin and precipitin titers gave the same amount of protection (I No. 6 to 10). On the other hand, serums that were poor in both agglutinins and precipitins were also poor in protective power (I No. 12 to 15).

The five serums that were tested with both Types I and III cultures differed in three instances in their protective power against the two types. This suggests that in standardizing serums it might be well to test against more than one type of culture unless a major strain could be found that would be representative of the different types of meningococci.

TABLE II

MENINGOCOCCUS MOUSE PROTECTION TESTS. SERUM TITRATIONS FOR AGGLUTININS, PRECIPITINS, AND FOR PROTECTIVE POWER AGAINST VIRULENT CULTURES

TEST CULTURE	POLYV. SERUM		AGGLUT. TITER	PRECIP. TITER		MOUSE PROTECTION						MOUSE PROT. DOSES PER C.C.	UNITS* PER C.C.
						SERUM DOSAGE—PERCENTAGE MICE SURVIVED							
	NO.	PREP.	TYPE III	TYPE III	POLYV.	0.05 C.C. PER CENT	0.01 C.C. PER CENT	0.005 C.C. PER CENT	0.002 C.C. PER CENT	0.001 C.C. PER CENT	0.0008 C.C. PER CENT		
23047 Type III Dose 200 million cocci[1]	1	304S	1000	60	120			100		100	80-100[2]	1300	130
	2	42	4800	15	15			100		80-100[2]	80	1250	125
	3	L526H	1800	60	120					100		1000	100
	4	024665	2000	80	120				100	80	0†	1000	100
	5	L527L	2000	80	160				100	60		740	74
	6	195	1600	60	30			100		40-60[2]		500	50
	7	194	2000	40	40			100		40-60[2]		500	50
	8	169-1C	400	60	180			100		40		500	50
	9	47	3200	32	60			100		40		500	50
	10	L518X	1200	60	60				100	40		500	50
	11	45	6400	16	30			100		20		250	25
	12	30M	800	10	15		80-100[2]	20		0	0	100	10
	13	EL1019	800	20	30		80	60		0		100	10
	14	201	400	8	20		80	60		20		100	10
	15	202	400	6	20		80	40		20		100	10
24995 Type I Dose 200 million cocci[1]	1	L527L	6400	80	160			100		60		550	55
	2	L518X	3500	60	60			100		60		550	55
	3	024665	4000	60	120			100		40		500	50
	4	42	9600	15	15		100	100		20		250	25
	5	L526H	4800	60	120			80		20		200	20
	6	43	3200	16	10	80	0					20	2

[1]Fifty million cocci gave 80 to 100 per cent mortality.
[2]Repeated tests.
*A unit = ten times the smallest amount of serum that will protect 80 per cent of the mice against a standard amount of virulent culture.
†The amount of serum in this test was 0.0005 c.c.

SUMMARY

Using a slight modification of Miller's method the virulence for mice of two meningococcus cultures Types I and III was increased so that a minimum fatal dose was obtained, a multiple of which was then used to test the protective power of antimeningococcus serums.

Sixteen different preparations of antimeningococcus therapeutic serums were titrated for their ability to prevent death in mice as well as for their agglutinin and precipitin titers.

It was found that the agglutinin and precipitin titers of the serums did run parallel with their protective power although in most instances the highest protection was obtained with the serums that were high in agglutinins or precipitins. The serums that were low in agglutinins and precipitins were, with the exception of one serum, also low in protective power.

The protective power of the serums differed when tested with Type I and Type III.

CONCLUSION

From the results obtained in this experiment the indications are that the mouse-protection test can be used to standardize the potency of antimeningococcus therapeutic serums. By means of this test a definite unitage of the protective power of the serums could be established which seems reasonable to assume would be a better index of their therapeutic power than the determination of their antibacterial antibody content.

It is suggested that the unit of the protective power of antimeningococcus serum should be ten times the smallest amount of serum which will protect for forty-eight hours 80 per cent to 100 per cent of a series of 5 to 10 mice against a multiple of fatal doses of virulent meningococcus cultures. By adopting a definite unitage in standardizing different preparations more uniform results could be obtained in different laboratories.

A serum of standard unitage should be used as control when new serums are standardized for protection. Such a serum could perhaps be supplied by the Hygienic Laboratories in Washington, D. C., and would serve as control of the validity of the tests.

Further work is in progress to increase the virulence of other types of meningococci and to test immune serums for cross protection against the different types of meningococci; also to find if possible, a major strain that could be employed in the routine testing of the potency of immune serums.

NOTE: Since the completion of this paper Miller[4] and Rake[5] published the results of similar investigations in which they confirmed the value of the mouse-protection test in standardizing antimeningococcus serums.

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A RAPID SLIDE TEST FOR THE SEROLOGIC DIAGNOSIS OF TYPHOID AND PARATYPHOID FEVERS*

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FELIX'S[1] contention that antigens of the proteus, typhoid, and paratyphoid group consist of two distinct fractions: the stable, somatic "O" antigen and the labile, flagellar "H" antigen, each giving rise to a corresponding agglutinin aroused new interest in the diagnosis of the enteric fevers. The existence of the H and O antigens as separate entities is questioned by Craigie,[2] but from a purely diagnostic standpoint it is relatively unimportant which theory is accepted since the injection into animals of a motile strain of an organism of the enteric group produces an antiserum in which the two types of agglutinins may be demonstrated. In the typhoid and paratyphoid groups the H agglutinin is type specific whereas the O agglutinin is group specific. According to Felix, the O agglutinin is indicative of infection while the H agglutinin shows little or no relation to the actual course of the disease.

The high titers for H agglutinins found in the blood of individuals inoculated with T. A. B. vaccines is too well known to merit further comment, but the height to which the O agglutinin titer may rise is not so clear. Felix[3] and Stuart and Krikorian[4] were unable to demonstrate O agglutinins in the blood after prophylactic immunization. On the other hand Gardner,[5] Smith[6] and Wyllie[7] found certain vaccinated individuals with O titers as high as 1:250. Recently Dennis and Berberian[8] demonstrated O agglutinins of relatively high titer in the blood of persons inoculated with T. A. B. vaccine. Furthermore they showed that "there was no correlation between the number of previous vaccinations, the interval of time since the last vaccination and the height of the titer, making the establishment of an arbitrary diagnostic titer impossible. Hence a single qualitative receptor analysis is not capable of differentiating between inoculation agglutinins and those due to infection."

A living motile culture can be used to detect the presence of both H and O agglutinins in a serum, whereas a single formolized or phenolized antigen cannot be used for this purpose. With the latter type of antigens there is an inhibition of O agglutination probably due to the organisms being kept apart

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mechanically by the hardened flagella, as pointed out by Craigie. In the Laboratories of the Connecticut State Department of Health living motile cultures have been used in the Widal test for several years and although the results of the tests are satisfactory there are certain technical disadvantages in this method. The strains used in the test must be checked for smoothness every three weeks; moreover, the antigens must be transplanted and standardized daily for use in the tests. It is apparent that all these technical procedures would be eliminated in a rapid slide test using concentrated H and O antigens.

The rapid slide agglutination test, for Brucella infection (contagious abortion in cattle), first demonstrated by Gwatkin,[9] was brought to its present state of reliability and accuracy by Huddleson.[10] Lienhardt and Kitselmann,[11] Palmer and Baker[12] and Welch and Mickle[13, 14] have emphasized the accuracy and efficiency of this test in diagnostic work. The rapid slide method proposed in this paper for the diagnosis of enteric fever follows in general the Huddleson technic.

PREPARATION OF ANTIGEN

Eberthella Typhosa Flagellate (HO).—A strain of *E. typhosa* of known antigenic make-up which has been carefully checked for smoothness is grown on agar pH 7.0 to 7.2 in Blake bottles for twenty-four hours at 37° C. The agar surface is then covered with not more than 5 ml. of 12 per cent NaCl containing 0.5 per cent formalin. After one or two hours the organisms may be washed from the agar by gently rocking the flasks. The suspension is then filtered through at least eight layers of cheesecloth and centrifuged at high speed in graduated centrifuge tubes for one to two hours to pack the organisms as completely as possible. The supernatant fluid is decanted and retained for later use, and the amount of packed organisms recorded. Just sufficient supernatant fluid is added to each centrifuge tube to pool the organisms. It was found that 5 to 7 ml. could be safely added to each 1 ml. of packed cells. Too much supernatant fluid added at this point will dilute the antigen to a degree where later titration is impossible without further concentration. The suspension is filtered through eight layers of cheesecloth and this highly concentrated suspension constitutes the antigen which is now ready for titration.

Both *Salmonella paratyphi* and *S. schottmuelleri* slide antigens are prepared in a similar manner. All three flagellate antigens are prepared in a similar manner. All three flagellate antigens (*E. typhosa*, *S. paratyphi*, and *S. schottmuelleri*) contain O as well as H antigen.

Eberthella Typhosa Somatic (O).—The same strain used in preparation of the flagellate (HO) antigen is used in making the O antigen and is carried out in a similar manner except that the organisms are grown for forty-eight hours at 37° C. rather than for twenty-four hours and are washed from the surface of the agar with 12 per cent NaCl rather than with the formolized salt solution. After centrifugation the supernatant fluid is discarded and the packed bacterial cells are pooled, using just sufficient 0.85 per cent salt solution for

transfer purposes. This concentrated suspension is placed in a large flask and 15 to 20 volumes of 95 per cent alcohol is added, the flask shaken vigorously for ten minutes and incubated at 37° C. for twenty-four hours.

At the end of the incubation period the antigen appears as a flocculent white mass from which considerable of the alcohol may be siphoned and discarded. The remaining alcohol is removed by centrifugation. About 5 to 7 ml. of 12 per cent salt is added for each 1 ml. of packed bacterial cells and the concentrated suspension is ready for titration.

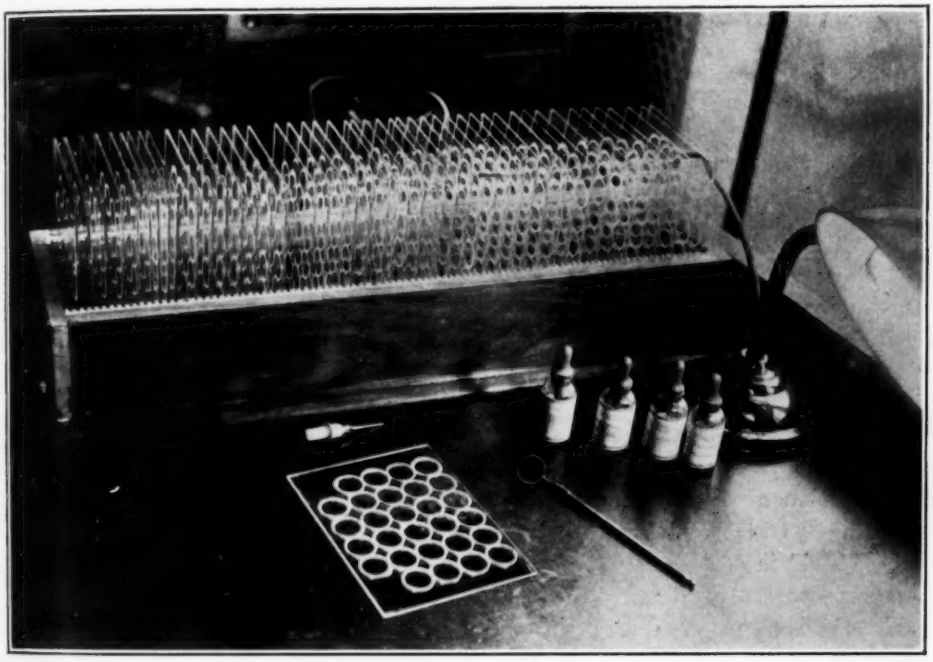

Fig. 1.—Showing slides with wax rings, four types of antigen used, wire loop and standardized Huddleson dropper.

MATERIALS

Standardized Dropper Pipette.—The standardized dropper pipette used for the slide test is similar to that described by Huddleson[10] and is prepared by drawing out thick-walled glass tubing of ⅛ inch bore and cutting the capillary end at 0.07 diameters (B and S gauge). Such a pipette delivers approximately 0.03 ml. per drop. A Kahn pipette may be used although the Huddleson dropper is more convenient.

Glass Slides.—The glass slides are made from ordinary picture frame glass 7 inches long by 5 inches wide and 1/16 inch thick. Twenty-eight one-inch wax rings are placed on the slide; four rows of six rings and one row of four, using a mixture of 70 per cent paraffin (m.p. 48° C.) and 30 per cent petrolatum heated to 130° to 140° C. The wire loop used to make these rings is prepared by winding tightly No. 28 gauge wire around a one-inch test tube. The loop formed in this manner is wound (single thread) with No. 12 thread and forced

into a regular platinum loop holder. The loop is dipped into the hot wax petrolatum mixture and then placed on the glass slide. With very little practice a single slide can be prepared in one minute. The first two rows of rings are used for O and H (*E. typhosa*) antigen, the third row for *S. paratyphi*, the fourth row for *S. schottmuelleri*, and the fifth row for controls on each antigen. We found it convenient to prepare 50 slides at one time for our investigational work, although it is probable that for routine diagnostic purposes only a few slides prepared in advance would be necessary. Antigen controls are necessary only on the first slide test made each day. Shortly after slides have been read they should be washed off under hot, running water. This removes both the wax rings and the serum antigen mixture. To clean the slides they are rubbed on both sides with Bon Ami, dried and wiped off with a clean cloth. Just before the wax rings are placed on the slides, it is desirable to go over each slide once with a piece of chamois.

ANTIGEN TITRATION

In titrating these antigens the desired dilution to be obtained is one that will show an agglutination on the slide which agrees with a similar dilution previously obtained in the tube test. Because the usual range of dilutions used in most macroscopic tube tests is 1:20, 1:40, 1:80, etc., it seemed advisable to concentrate the slide antigens to the point where agreement would be obtained with these dilutions in a tube test. The tube test used for comparison utilized 0.5 ml. of the serum dilutions and 0.5 ml. of antigen. Negative, partial positive and strongly positive agglutinating serums are used for the titration of all antigens. In each of four test tubes is placed 0.5 ml. of the antigen to be titrated, and 0.1, 0.2, 0.4, and 0.5 ml. of the retained supernatant fluid is added. In titrating O antigen 12 per cent salt is used since this supernatant fluid contains flagellated organisms. The diluted antigen in each tube is then tested with serums of known tube test titer, using one drop (0.03 ml.) of antigen and 0.08, 0.04, 0.02, 0.01, 0.005 and 0.002 ml. respectively of each known serum. These dilutions correspond to dilutions of 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 in the tube test used in these laboratories. The serum and antigen is mixed with pieces of wooden applicators or toothpicks starting with the 0.002 ml. amount of serum working toward the 0.08 ml. amount. The glass slide is then rocked back and forth (usually fifteen to twenty times) and the agglutination read at once by holding the slide over a desk lamp, so that the light is transmitted through the slide but not directly into the observer's eyes. The clumping of the organisms takes place at once with positive serums and it may be observed to increase as the rocking process is carried out. The amount of clumping is estimated as ++++ (complete), +++ (75 per cent), ++ (50 per cent), + (25 per cent), ± (faint to 25 per cent), and 0 (no clumping). The dilution of antigen that shows no clumping with negative serums, complete clumping with a clear background with strongly positive serums in all amounts of serum used and gives the titer with partial positive serums obtained in the tube test will indicate the proper dilution of the prepared antigen. The titers obtained with the slide test should agree with the titers obtained by tube test within a ± to + result. Supernatant fluid

is then added to the antigen in the amount indicated by titration. If considerable evaporation takes place when centrifuging, 0.85 per cent salt solution or distilled water should be used for the diluent, since too great a salt concentration adversely affects the dispersion of the antigen.

After titration, sufficient gentian violet and brilliant green are added to all antigens from 1 per cent aqueous stock solutions so that a final concentration of 1:40,000 is obtained with the former dye and 1:20,000 with the latter. These dyes prevent contamination of the antigens during use and facilitate the reading of tests. Five months after preparation the slide antigens prepared in these laboratories still give consistent results.

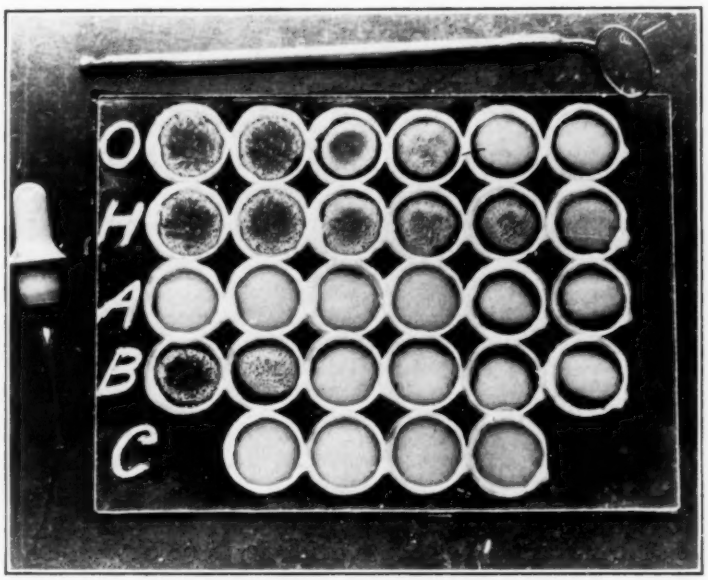

Fig. 2.—Showing typical agglutination with *E. typhosa* O and H antigen, negative *S. paratyphi*, cross reaction with *S. schottmuelleri* and controls on each antigen.

TECHNIC OF TEST

In the routine diagnostic test we have found it more convenient to use all serum amounts described above except the 0.002 ml., i.e., 0.08, 0.04, 0.02, 0.01, and 0.005 ml. of serum giving dilutions corresponding to 1:20, 1:40, 1:80, 1:160, and 1:320 in the tube test. The serum to be tested is pipetted in the above amounts with a Kahn pipette (0.2 ml. graduated in hundredths) into four rows of rings on the glass slide starting with the greatest amount and going from left to right. In the fifth row (used only for the first test each day) 0.08 ml. of 0.85 per cent salt solution is added to each of the four rings. A drop of O antigen is added to each of the serum amounts in the first row and to the first ring in the fifth row (control). Similarly, a drop of H antigen is added to each ring in the second row and the second ring in the fifth row. *S. paratyphi* antigen is added to the third row and *S. schottmuelleri* to the fourth row, each with the appropriate controls in the third and fourth rings respec-

tively in the fifth row. All antigens are shaken gently but well before using. Each row of serum antigen mixture is mixed thoroughly with a separate toothpick or piece of applicator starting with the smallest amount (0.005 ml.) of serum working from right to left.

After mixing, the glass slide is gently rocked back and forth 15 or 20 times. (We usually check the degree of clumping after each five.) The degree of clumping is estimated as noted previously and recorded. The type of clumping obtained with the slide test O antigen does not correspond to the typical small-flaking or granular agglutination obtained in the tube test, and hence O and H agglutination cannot be differentiated by appearance. This is no disadvantage since both types of antigen are used.

DISCUSSION

Since the slide test has been developed, 256 serums from patients suspected of having typhoid fever and 200 Wassermann serums have been studied by this method, and the results compared with the macroscopic tube test. In these laboratories a live motile antigen is used in the tube test which is incubated three hours at 56° C. and read after being placed in the refrigerator overnight. Through the courtesy of the New York State Laboratories it was possible to study a further group of 200 Wassermann serums, using the New York *E. typhosa* H and O antigens.

Of the total of 456 serums studied with the slide test and compared with the Connecticut method, excellent agreement was obtained. The slide antigens were slightly more specific, in that fewer questionable reactions were obtained in known negative serums. Inasmuch as the Connecticut method does not utilize a pure O antigen it seemed advisable to compare the O and H slide antigens with the New York O and H antigens following the New York tube test technic. In a series of 200 Wassermann serums the New York O antigen showed agglutination in 11 per cent, the New York H antigen in 33 per cent, whereas the slide O antigen showed some reaction in 9 per cent and the H in 43 per cent. The degree of agglutination with the New York H antigen was somewhat greater than the slide H antigen. Since the slide antigens were standardized originally against the Connecticut tube test antigen, it would appear that these results are in reasonably close agreement.

SUMMARY

A rapid slide test is proposed for the routine diagnosis of typhoid and paratyphoid fevers. This test makes use of concentrated antigens and undiluted serum and may be carried out within a few minutes. Comparisons made with standard routine diagnostic tube tests indicate that the rapid method is as accurate and as specific as the tests with which it was compared.

The authors wish to express their appreciation to the Misses Betty Robinton and Sarah B. Whitney for technical assistance.

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A RAPID METHOD FOR PREPARING ANTIGENS FOR THE WASSERMANN REACTION*

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THE preparation of an antigen for the Wassermann reaction is generally a time-consuming procedure. There have been reported several rapid methods for preparing antigens. Ecker and Sasano (1919) published the results of their method and concluded that suitable antigens could be made by extracting normal heart tissue for one to three hours with boiling alcohol in a reflux condenser. These antigens "had more or less marked fixing power at as high a dilution as 1-200." Kolmer (1928) reported his rapid method for preparing antigen. He extracted 25 gm. of beef heart powder with 200 c.c. of ether in an Erlenmeyer flask fitted with a Leibig condenser, boiling two hours. This was followed by extracting the dried powder by boiling for two hours with 95 per cent ethyl alcohol and finally with absolute ethyl alcohol. The method was similar to his original method (1922) except boiling was employed instead of the usual extraction in the incubator for several days. He found that the shorter method using boiling alcohols may sometimes yield extracts of lesser antigenic sensitiveness, but on the whole were serviceable and satisfactory.

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Several years ago necessity arose for the preparing of an antigen for the Wassermann test in a short period of time. Kolmer's (1922) antigen had been used in this laboratory for a number of years with good results. In order to save time antigen was prepared according to Kolmer's method with the exception that all extractions were carried out in a Soxhlet extraction apparatus. The antigen proved to be very satisfactory having a high antigenic unit and very low anticomplementary and hemolytic units. There was little difference between the antigen prepared by the rapid method and one prepared according to Kolmer's method. In fact, the antigen by the rapid method gave a little higher antigenic unit than one prepared by his original method and the anticomplementary and hemolytic units were identical. Further work on preparing antigens by the use of Soxhlet apparatus was continued and the following method has produced satisfactory antigens:

PREPARATION OF ANTIGEN

1. Twenty-five grams of beef heart powder (Difco) are extracted with approximately 150 c.c. of ether for four or five hours in a Soxhlet apparatus. The time depends upon the rate of percolation. Usually the apparatus will flush every three to five minutes.

2. The ether extract is placed in a tightly stoppered bottle and stored in the refrigerator.

3. The thimble containing the beef heart is removed from the Soxhlet apparatus and thoroughly dried. The Soxhlet apparatus is also dried.

4. The beef heart is then extracted in the Soxhlet with 95 per cent alcohol for four or five hours.

5. The alcoholic extract is placed in an evaporating dish and fanned dry. The residue is dissolved in 25 or 30 c.c. of ether and allowed to stand about two hours. The ether is then decanted and mixed with the ether of the primary extraction.

6. The ether extract is concentrated to about 25 or 30 c.c. and about 150 c.c. of C.P. acetone added. The precipitate which forms is permitted to settle and the acetone poured off. Fresh acetone is placed in the dish containing the precipitate. This acetone is also discarded and the acetone insoluble precipitate freed of acetone. The acetone insoluble lipoids and 0.2 gm. cholesterol are dissolved in approximately 25 c.c. of ether and added slowly to 100 c.c. of absolute ethyl alcohol, agitating constantly.

7. The antigen is placed in the 37° C. incubator for approximately twelve hours when it is removed and placed in the refrigerator for twenty-four to forty-eight hours. It is then filtered through fat-free filter paper and is ready for titrating.

It will be noted that the heart powder is not extracted with absolute alcohol. The extraction of the heart powder with 95 per cent alcohol in a Soxhlet apparatus gives approximately the same results as when absolute alcohol is used. Antigens have been prepared by extracting the heart muscle with 95 per cent alcohol followed by another extraction with absolute alcohol. No appreciable difference could be noted in these antigens and those that were extracted once with 95 per cent alcohol.

TITRATION

The protocol of a typical titration is given in Tables I, II, and III.

TABLE I

ANTIGENIC TITRATION

ANTIGEN 0.5 C.C.	POOLED POSITIVE SERUMS (1-10)	COMPLEMENT (1-30) 2 UNITS	SALINE	Refrigerator 18-20 hours and 10 minutes in 37° C. water-bath	SHEEP'S AMBOCEPTOR 2 UNITS	SHEEP'S R.B.C. 2%	RESULTS AFTER 1 HOUR IN 37° C. WATER-BATH
1- 300	0.5	1.0	0.1		0.4	0.5	No hemolysis
1- 400	0.5	1.0	0.1		0.4	0.5	No hemolysis
1- 500	0.5	1.0	0.1		0.4	0.5	No hemolysis
1- 600	0.5	1.0	0.1		0.4	0.5	No hemolysis
1- 800	0.5	1.0	0.1		0.4	0.5	No hemolysis
1-1000	0.5	1.0	0.1		0.4	0.5	No hemolysis
1-1200	0.5	1.0	0.1		0.4	0.5	No hemolysis
1-1600	0.5	1.0	0.1		0.4	0.5	No hemolysis
1-2000	0.5	1.0	0.1		0.4	0.5	No hemolysis
1-2400	0.5	1.0	0.1		0.4	0.5	No hemolysis
1-3200	0.5	1.0	0.1		0.4	0.5	Slight hemolysis
1-4000	0.5	1.0	0.1		0.4	0.5	Complete hemolysis
Control	0.5	1.0	0.6		0.4	0.5	Complete hemolysis
Control	0.0	1.0	1.1		0.4	0.5	Complete hemolysis

TABLE II

HEMOLYTIC TITRATION

ANTIGEN 0.5 C.C.	POOLED NEGATIVE SERUMS HEATED (1-10)	SALINE	Refrigerator 18-20 hours and 10 minutes in 37° C. water-bath	SHEEP'S R.B.C. 2%	RESULTS AFTER 1 HOUR IN 37° C. WATER-BATH
1- 4	0.5	1.5		0.5	No hemolysis
1- 5	0.5	1.5		0.5	No hemolysis
1- 6	0.5	1.5		0.5	No hemolysis
1- 8	0.5	1.5		0.5	No hemolysis
1-10	0.5	1.5		0.5	No hemolysis
1-12	0.5	1.5		0.5	No hemolysis
1-16	0.5	1.5		0.5	No hemolysis

Hemolytic unit: No hemolysis in 1-4 dilution

TABLE III

ANTICOMPLEMENTARY TITRATION

ANTIGEN 0.5 C.C.	POOLED NEGATIVE SERUMS HEATED (1-10)	COMPLEMENT (1-30) 2 UNITS	SALINE	Refrigerator 18-20 hours and 10 minutes in 37° C. water-bath	SHEEP'S AMBOCEPTOR 2 UNITS	SHEEP'S R.B.C. 2%	RESULTS AFTER 1 HOUR IN 37° C. WATER-BATH
1- 4	0.5	1.0	0.1		0.4	0.5	Slight hemolysis
1- 5	0.5	1.0	0.1		0.4	0.5	Slight hemolysis
1- 6	0.5	1.0	0.1		0.4	0.5	Almost complete hemolysis
1- 8	0.5	1.0	0.1		0.4	0.5	Complete hemolysis
1-10	0.5	1.0	0.1		0.4	0.5	Complete hemolysis
1-12	0.5	1.0	0.1		0.4	0.5	Complete hemolysis
1-16	0.5	1.0	0.1		0.4	0.5	Complete hemolysis
Control	0.5	1.0	0.6		0.4	0.5	Complete hemolysis
Control	—	1.0	1.1		0.4	0.5	Complete hemolysis

The results of the titration of this antigen, which, by the way, is very typical of all the antigens produced by this method, show that the anticomplementary unit was low, the antigen was not hemolytic in 0.5 c.c. of 1-4 dilution and the antigenic unit was fairly high, 0.5 c.c. of 1-2,400 dilution. From these titrations

the conclusion might be drawn that this was a good antigen, but there is one point which is very important and should always be considered, and that is, is the antigen sensitive, and is it specific? To prove that the antigen would meet these qualifications it has been employed in the laboratory for a number of years and approximately 50,000 Wassermann tests run. These Wassermann tests were checked with the Kahn test and the results agree remarkably well. It should be stated, however, that the Wassermann tests using this antigen are considered by this laboratory to be more reliable than the Kahn reaction. Statements of physicians in this state have shown that the Wassermann test as run in this laboratory correlates exceptionally well with the clinical history of the patients.

CONCLUSIONS

1. The Soxhlet apparatus is a very efficient and rapid method for extracting the soluble lipoids from beef heart.

2. The time for extracting the lipoids from the beef heart is only eight to ten hours by this method compared with fifteen days by Kolmer's method.

3. Antigens produced by this method are of high antigenic titer and have low anticomplementary and hemolytic units.

4. The antigens have good specificity as determined by comparison with the Kahn precipitation test on at least 50,000 serums.

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DARK-FIELD ILLUMINATION IN THE DIAGNOSIS OF TUBERCULOSIS AND MALARIA*

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DARK-FIELD illumination for the study of living organisms has become an accepted practice. Its value in the study of stained films of sputum and blood seems to deserve more attention than it has received. After a little experience with the technic, the search for tubercle bacilli not only takes less time, but there is less likelihood of missing a few scattered organisms, as shown by Schoenheit.[1] The same thing seems to be true of the malaria plasmodium. In both cases the dark-field observation should be confirmed by changing to ordinary

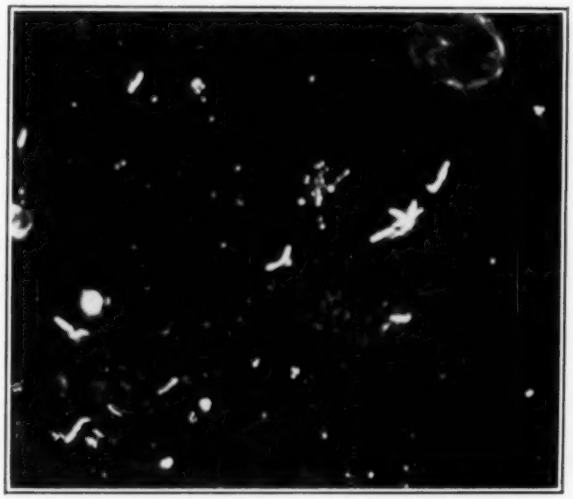

Fig. 1.—Tuberculous sputum with dark-field illumination. The bacilli are conspicuous, but the pus cells are almost invisible. Dark field broadens the image of the bacteria.

transmitted light, using ground glass or smoked glass to reduce the light intensity.

Dark-field examination of tuberculous sputum shows the fuchsin-stained bacilli brightly fluorescent (Fig. 1) while the blue-stained bacteria and pus cells are very inconspicuous. A change to transmitted light shows the red bacilli even with a 4 mm. dry objective, but the oil immersion lens can then be used to verify the details (Fig. 2). In a Giemsa stained blood film the malaria plasmodium is also easily found under dark field, excepting the small ring forms. Here again it is necessary to turn to transmitted light before making a diagnosis. It is, therefore, desirable to be able to change from dark field to light field without much manipulation.

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Most laboratory microscopes can be adapted for dark field with very little expense aside from a powerful light, such as a 6 volt, 108 watt headlight lamp[2] mounted with a condensing lens. Such a light is very satisfactory for alternating current, but for direct current a pointolite lamp, or other powerful source, might be better.

Using a two lens Abbe condensor, N. A. 1.20, and a X40 (4 mm.) objective, N. A. 0.65, it is possible to get dark-field illumination by the use of a center

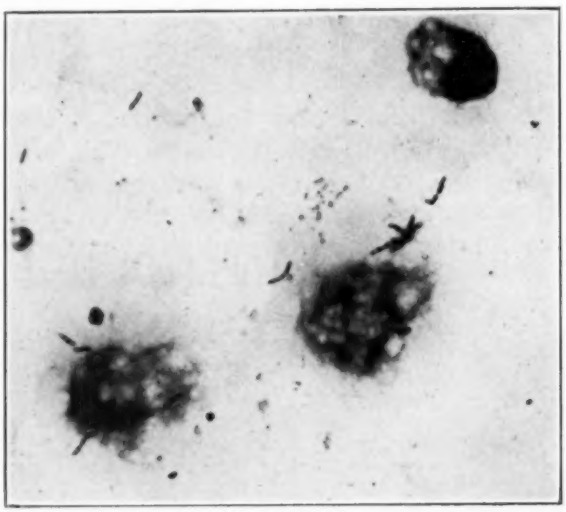

Fig. 2.—Tuberculous sputum with transmitted light. The pus cells are the most conspicuous objects.

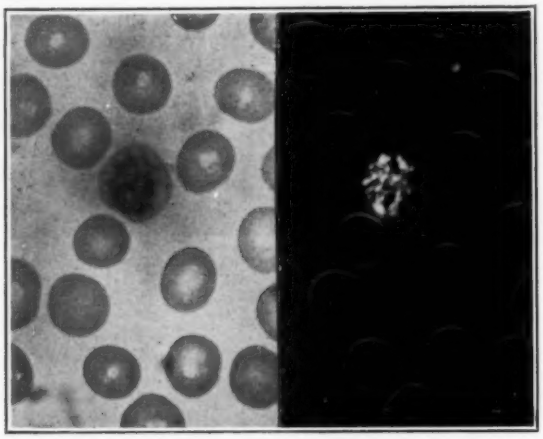

Fig. 3.—Malaria plasmodium in blood cell. Left, as seen with bright field. Right, with dark field.

stop (Fig. 4) below the condensor.[3] If the condensor and slide are connected with a drop of water (oil is too messy), a better dark field may be obtained; but when searching a large film, the dry condensor has the advantage of not getting water into the mechanism of the mechanical stage.

The required size of the center stop depends on the aperture of the objective and on the ocular that is being used. It is well, therefore, to have different

sizes, preferably of metal, but stops for experimental use can be cut out of heavy black paper or thin cardboard. The object slide must be thin enough to permit the substage condensor to focus its outer rays on the film. Some condensors require slides not more than 0.8 mm. thick; in any event really thick slides cannot be used.

Dry objectives are very sensitive to incorrect cover glass thickness in dark-field work, so it is better to use only cover glasses between 0.15 and 0.20 mm. thick. The cover glass can be mounted on the film with paraffin oil or cedar oil; or with balsam if a permanent preparation is desired.

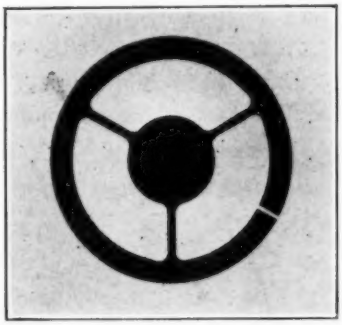

Fig. 4.—Center stop for use below condensor to produce dark field. Several sizes should be available, such as 12, 16, 18, and 20 mm. diameter. The outside diameter, of course, must fit the microscope in use.

Fig. 5.—Visualizing the rays by placing a block of uranium glass on the upper surface of the condensor. The left figure shows the full cone of light with ordinary bright-field illumination. The center figure shows a hollow cone of light produced with a small center stop. The right figure shows the effect of a large center stop.

One method of testing the corrections of microscope objectives is by use on a dark-field specimen. If, therefore, the objective gives defective images, from rough usage, dirty lenses, etc., good definition cannot be expected by this method.

As in all dark-field work, the cover glass must be clean, for every dust particle is conspicuous under this method of illumination. To clean cover glasses, Stitt's method[4] is good. Immerse the cover glasses in "Bon Ami" (5 per cent in water) and allow them to dry. Just before use wipe off the Bon Ami. Of course, in using a high power dry lens a cover glass is essential, but those who have a X20 apochromatic objective should try Coles' method[5] of working without a cover glass, by increasing the length of the microscope tube.

So far it has been assumed that the simplest outfit, only, is at hand. But if the equipment includes a modern oil immersion objective equipped with an iris diaphragm between the lenses, so that the aperture can be reduced to 0.65, it can be used for the dark field, as well as transmitted light, without a cover glass. Of course, the diaphragm should be opened for bright-field study.

Sometimes a green color screen is useful, to remove the secondary spectrum of achromatic objectives. It should be a very transparent green and can be purchased, or made with the dye called "rapid filter green."

This article should not close without mentioning Rheinberg's differential color illumination.[6] By using a dark colored center disc (blue, let us say) and a lighter colored rim (red) the objects on the slide will be colored red while the background is blue.

This method is a beautiful demonstration of the theory of dark-field illumination. Recently, under the name Mikropolychromar, there has been introduced a very accurate, but expensive, attachment for producing this "optical staining."

I take this opportunity to express my appreciation to Craig Howard, of the Department of Bacteriology, University of Cincinnati Medical Department, for placing his many interesting slides at my disposal.

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BLOOD CALCIUM DETERMINATION, USING STANDARD CALCIUM CHLORIDE SOLUTION*

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THE normal blood calcium value is very constant: from 9 to 11 mg. per 100 c.c., in children slightly higher, a very close range; yet it is important to know whether in a given patient it is below or over these values, especially in judging borderline cases in which calcium therapy is contemplated.

Five-tenths milligrams, certainly as much as 1 mg. difference in results over or under that actually present, especially when calcium is found to be below the minimum normal, is of utmost importance to the physician in judging the necessary treatment, particularly in view of therapeutic claims made for certain calcium salts. Erroneous results lead the physician to conclude that the prescribed medication was valueless. In view of the narrow normal threshold, wrong determinations handicap establishment of a diagnosis and line of treatment. A difference of 0.05 c.c. to 0.1 c.c. in a deteriorated 0.01 N potassium permanganate solution will show a difference of 0.5 to 1.0 mg. of calcium per 100 c.c. of blood, an error too great.

The most widely employed methods for determination of blood calcium are those of Kramer and Tisdale, and Clark's modification of the former, in both of which 0.01 N potassium permanganate solution is used to titrate in the presence of sulphuric acid the calcium oxalate formed after precipitation with ammonium oxalate. The discrepancies above stated are traceable largely to errors in preparation and standardization of solutions, for too often there is a tendency to assume that the 0.01 N potassium permanganate has remained unchanged since previous determination. We found that volumetric solutions of potassium permanganate had to be checked for each determination; and the ammonium oxalate and oxalic acid used to standardize the permanganate solution also had to be restandardized, although it now is possible to obtain very pure ammonium oxalate and oxalic acid. When it is considered that very small quantities of calcium are dealt with, even the slightest change in titer suffices to offset the result.

As Redwood and Myers indicate, the method of Kramer and Tisdale requires a highly refined technic. In the hands of chemists trained in quantitative procedure and methods of standardization, accurate results are not difficult to obtain. The vexing fact, however, is that application of the methods elaborated by specialists in biologic chemistry often is left to technicians who, though highly conscientious, lack the chemical knowledge of basic principles concerning reactions, standardization and familiarity with quantitative procedure. Owing to insufficient technical training, results as high as 15 to 16 mg. calcium per 100 c.c. of blood and as low as 4 to 6 mg. in normal cases have been reported in institutions of

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no small standing, despite the fact that the contrary was indicated and later proved to be so. In one hospital laboratory, potassium permanganate solution was purchased and used during an entire month and not restandardized during the interim. The calcium determinations made under such circumstances were so unreliable that, in view of the treatment the patient received, physicians in the pediatric department were at a loss to interpret them.

The methods used for blood calcium determination are somewhat tedious, and in laboratories where only an occasional determination is made, the discrepancy between duplicates may run as high as 1 mg.

In addition to deterioration of 0.01 N potassium permanganate solutions, other factors tending to vitiate results are:

Incomplete washing of the calcium oxalate precipitate, leaving traces of ammonium oxalate, thereby increasing the result.

Incorrect calibration of pipettes used in drawing up the serum; improper correlation between pipettes, burets, and volumetric flasks employed in making 0.01 N potassium permanganate solution from the 0.1 N.

Incomplete centrifugation of the calcium oxalate precipitate and insufficient time allowed for precipitation. Even though one-half hour is given in the method, higher results are obtained if the precipitate is allowed to form in the ice box for several hours or overnight.

Occasionally, unknown to the worker, the wrong anticoagulant is used (potassium or sodium oxalate). Of course, in this case the determination is valueless.

The following modified method is offered to those who have encountered the difficulties above noted, and not to supplant the well-known method of Kramer and Tisdale. Preparation and standardization of 0.01 N potassium permanganate are eliminated without loss of accuracy. The method will find easy application where only occasional calcium determinations are made and facilities are inadequate. It is based upon an easily prepared standard calcium chloride solution which keeps indefinitely. With each blood calcium determination a similar quantity of standard calcium chloride solution is used. The serum and calcium chloride solution are run simultaneously, both being treated alike. The standard calcium chloride solution, containing 0.1 mg. per c.c., serves to give the value of permanganate calcium, from which the serum calcium can be calculated.

Solutions Required.—1. *Standard Calcium Chloride Solution:*

Dissolve 0.2498 gm. of pure calcite (calcium carbonate) Baker's in a little dilute HCl in a wide evaporating dish of 50 to 100 c.c. capacity, care being taken to avoid loss by spattering: (Run the acid down the side of the dish, allowing the reactions to proceed slowly). Carefully evaporate the solution several times to near dryness, each time adding distilled water, and last, evaporate to near dryness, expelling the last traces of HCl. Dissolve the residue in distilled water and dilute to 1 liter; 1 c.c. of this solution equals 0.1 mg. of calcium.

2. *Potassium Permanganate Solution:* Dissolve about 0.030 to 0.040 gm. of potassium permanganate crystals in 100 c.c. of distilled water. Keep in dark bottle. This solution ***need not be standardized.*** (A few crystals, the size of

caraway seed, dissolved to a solution sufficiently transparent to read print through, have been found convenient.)

3. *Three Per Cent Ammonium Oxalate Solution.*

4. Approximately N/1 sulphuric acid: 28 c.c. H_2SO_4 added to 970 c.c. of distilled water.

5. *Two Per Cent Ammonia Water:* If sodium citrate is used as anticoagulant, correction for the dilution should be made when final calculation is completed. (Textbooks omit this statement, suggesting that 1 c.c. of saturated sodium citrate solution can be used to 10 c.c. of blood.)

Procedure.—Place in a conical centrifuge tube 0.5 c.c. of ammonium oxalate solution and 2 c.c. of citrated plasma. Then add another 0.5 c.c. of ammonium oxalate solution and make up to 6 c.c. Mix* thoroughly with distilled water and let stand for one-half hour. Allowing the precipitate to form in the ice box overnight is recommended by other workers, as it yields higher results. This we also have found to be the case. Centrifugalize at moderate speed (1,500-1,800 r.p.m.) until sedimentation is complete and carefully pipette off the supernatant fluid, leaving 0.3 c.c. in the tube. After the precipitate is *well packed* in the tube apex we have found that the supernatant fluid can be decanted and the tube drained in the upright position, mouth downward upon filter paper. The precipitate is retained undisturbed.† Repeat the washing *not less than three times,* using about 3 to 4 c.c. of ammonia water, thoroughly stirring the precipitate by rotary motion of the tube. Use no stopper! After each washing centrifugation should be continued until the precipitate is firmly packed in the conical tube end. Last, dissolve the precipitate in 2 c.c. of N/1 sulphuric acid by placing in a water-bath and heating to 75° C. Simultaneously, employ 2 c.c. of calcium chloride solution and treat exactly the same as the serum.

Titrate with potassium permanganate solution made for the purpose. It is best to titrate the standard calcium chloride solution first to a faint pink and then match this tint with that obtained in titrating the calcium serum.

Calculation:

$$\frac{C \times S}{P} \times 50 = \text{serum calcium.}$$

C = mg. of calcium in 2 c.c. of standard calcium chloride solution (0.2 mg.).
S = c.c. of $KMnO_4$ used in titrating the serum.
P = c.c. of $KMnO_4$ used in titrating the standard calcium chloride solution.

Example:

1.2 c.c. of potassium permanganate solution used for 2 c.c. of standard calcium chloride solution = P.
1.15 c.c. of potassium permanganate solution used for 2 c.c. of serum = S.
2 c.c. of standard calcium chloride solution contains 0.2 mg. of calcium = C.
$0.2 \times 1.15 \div 1.2 \times 50 = 9.58$ mg. calcium per 100 c.c. of blood.

CONCLUSIONS

1. Accurate, dependable and easily performed method. Steps simplified.

2. The potassium permanganate solution used *need not be standardized.* It

*Adding the oxalate solution first and last permits thorough admixture of serum and oxalate; otherwise, much more time is required uniformly to mix the liquids.

†Tisdale used this simplified procedure with a centrifuge tube not over 6 to 7 mm. at the 0.1 mark. Centrifuge tubes with wide apices should not be used.

can be made just prior to a given determination and used immediately. Other volumetric solutions heretofore employed, dispensed with.

3. The principle of this method is based upon a standard calcium chloride solution, easily made, and keeps indefinitely.

4. Of value where insufficient blood has been drawn for duplicates, as in children.

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A MECHANICAL DEVICE FOR PREPARING FINE SUSPENSIONS OF TUBERCLE BACILLI AND OTHER MICROORGANISMS*

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THE preparation of fine suspensions of tubercle bacilli is encumbered by many detailed obstacles which retarded accurate quantitative studies with these bacilli for a great many years. In retrospect, one is reminded of the schemes which involved filtration, centrifugation, and numerous other procedures and which left the experimentalist with a feeling of uncertainty as to the actual amount of bacilli present in completed suspensions. Finally, after 1918,[1] reproducible suspensions of tubercle bacilli were prepared by us[2] by slow and laborious manual manipulation, which revealed the presence of about a billion bacilli per milligram moist weight of culture. This method was adhered to until quite recently when it was found that the addition of a few drops of 0.5 per cent solution of sodium taurocholate (because of its surface tension lowering properties[3]) in initiating grinding would materially assist in speeding the operation and give more consistent results for obtaining fine suspensions. With this improvement, obviating the frequent tendency to lumping of the bacilli on the first addition of liquid (0.9 per cent sodium chloride or other watery solutions), the method as now recommended is briefly: about 10 mg. of moist young culture (about three to five weeks old) is accurately weighed in a sterile graduated 15 c.c. centrifuge tube; the bacilli are carefully ground on the glass tube by means of a sterile rounded glass rod slightly smaller than the bowl of the tube; then one drop of sterile 0.5 per cent sodium taurocholate solution is added and the bacilli are thoroughly ground in this with a continual rotary motion; finally, 4 or 5 drops more of the taurocholate solution are added, a drop at a time after each grinding;

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after the grinding is completed, requiring usually five to ten minutes, 0.9 per cent sterile salt solution is added first by drops and mixed carefully with the bacilli and finally enough to give the desired concentration of the suspension. The final suspension from which further dilutions are to be made must possess a uniform milky opalescence without lumps or particles and contain a definite amount of bacilli per cubic centimeter. The dilutions are then made with sterile saline solution in stages of one to ten. In order to avoid contamination while manipulating the dilutions, it is well to have a separate small flask of saline solution for each dilution to be prepared. It is also

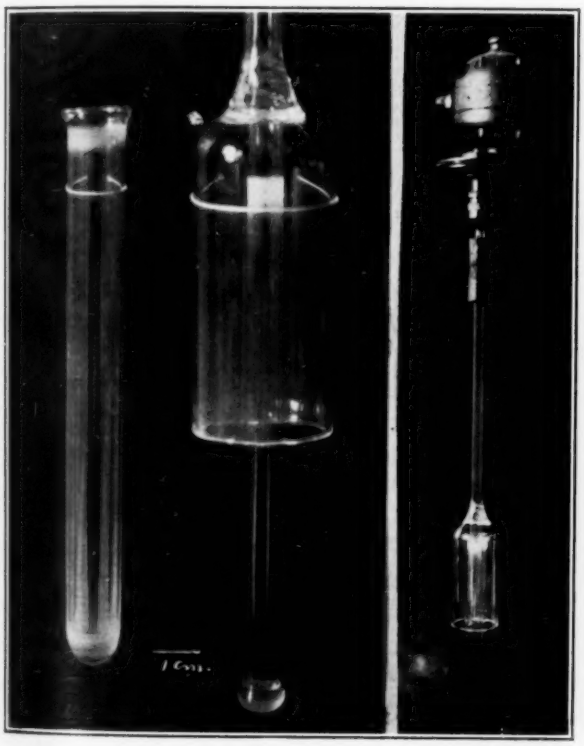

Fig. 1.

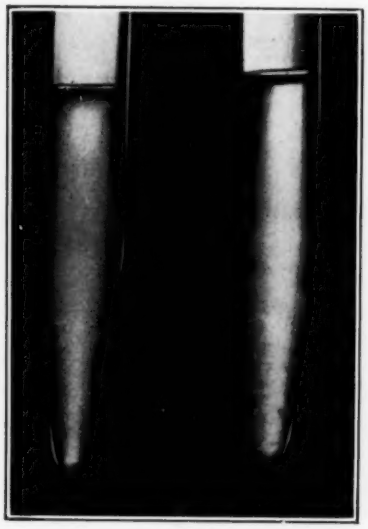

Fig. 2.

Fig. 1.—Shows the glass parts of the grinding apparatus at the left, and on the right with motor attached during the operation of grinding to make a fine bacillary suspension from a weighed amount of culture.

Fig. 2.—Photograph taken by transmitted light showing the condition of a satisfactory fine suspension of tubercle bacilli with no flocculation at this time, on the left; and an unsatisfactory suspension, on the right, examined six hours after they were prepared from the same culture and containing 1 mg. of bacilli per cubic centimeter. Note the definite flocculation of the bacilli in the unsatisfactory suspension which became evident one hour after standing.

advisable to use individual 1 c.c. sterile pipettes for each transfer, so that 1 c.c. is transferred to a 15 c.c. centrifuge tube, and to this is added the 9 c.c. of sterile saline solution.

It appeared that a further improvement in technic might result from the use of an apparatus devised for this purpose by Dr. Benjamin Sher of the City of Chicago Municipal Tuberculosis Sanitarium, who had not described it but had used it for grinding large amounts of tubercle bacilli. Dr. Sher

kindly submitted the original apparatus for our trial, on which plan the illustrated apparatus used in the tests reported below was built. He also consented to the publication of this description by us so that it might be available to others interested in making uniform suspensions of bacilli. As shown in the illustrations, the apparatus, entirely constructed of Pyrex glass, consists of a long glass tube (about 15 inches long) with pestle end ground carefully into the floor of a Pyrex test tube about six inches long and calibrated to 10 c.c. Above the height of the test tube length is welded a glass hood to prevent contamination. Actually in preparing this apparatus, the hood is inner sealed on the lower portion (pestle) and finally the upper glass tube attached.*

In using the grinding apparatus, it is sterilized either by dry heat or autoclaving. It is attached to a slowly revolving laboratory stirring motor by means of a piece of heavy wall rubber tubing. The bacilli to be ground are weighed in the bottom of the test tube, a drop of 0.5 per cent sodium taurocholate solution is added, and the mass is ground for two to five minutes, after which it is diluted as described for the manual procedure. In order to compare the results made by the manual procedure with that made with the motor, a series of suspensions of different cultures at different ages and from different strains of tubercle bacilli (human, bovine, and avian; virulent and avirulent) were compared by means of the growth limit culture test.[2] The results are presented in Table I.

TABLE I

THE EFFICIENCY OF THE MOTOR-DRIVEN GRINDER FOR PREPARING FINE SUSPENSIONS OF TUBERCLE BACILLI

STRAIN OF BACILLI‡ AND AGE OF CULTURE	CONCENTRATION OF BACILLI IN MILLIGRAMS PER CUBIC CENTIMETER SEEDED ON INSPISSATED EGG YOLK MEDIUM					
	10^{-4}*		10^{-6}*		10^{-8}*	
	MANUAL PREP.	MOTOR PREP.	MANUAL PREP.	MOTOR PREP.	MANUAL PREP.	MOTOR PREP.
Virulent human tubercle bacilli No. 7 (28 days)	3-3†	3-3	2-3	3-3	1-4	1-5
Avirulent human tubercle bacilli (19 days)	3-3	3-3	3-4	3-3	1-4	2-4
Virulent bovine tubercle bacilli No. 39 (38 days)	3-3	3-3	3-4	3-4	0	0
Avirulent bovine tubercle bacilli BCG (28 days)	3-3	3-3	3-4	3-4	1-6	1-5
Avian tubercle bacilli No. 3 (37 days)	3-3	3-3	3-4	3-4	2-4	3-5

*The amount of suspension per cubic centimeter is designated by the exponents as follows: $10^{-4} = 0.000{,}1$; $10^{-6} = 0.000{,}001$; and $10^{-8} = 0.000{,}000{,}01$ mg.

†The first numeral indicates the number of culture tubes (of a total of 3) positive and the second numeral, the number of weeks incubation after which the first positive culture appeared.

‡The tabulation is representative of the findings with about twelve different strains of bacilli tested in this study.

*We are indebted to Mr. L. D. Miller for assisting with the experimental work.

*The apparatus illustrated was made for us by Mr. George Reppert, glass blower at the University of Chicago.

The results recorded in Table I indicate that motor grinding can be employed with equal efficiency to manual grinding for the preparation of fine suspensions of tubercle bacilli. It is also noted, in conformity with previously recorded observations, that bovine tubercle bacilli do not lend themselves quite as well to obtaining fine suspensions as do cultures of human or avian tubercle bacilli or of BCG.

SUMMARY

The method of preparing reproducible graded fine suspensions of tubercle bacilli is described. A new apparatus for grinding these bacilli mechanically is presented which gives results equal to those obtained with careful manual grinding and is timesaving.

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MODIFICATIONS IN THE COLORIMETRIC DETERMINATION OF THE PLASMA PROTEINS BY THE FOLIN PHENOL REAGENT*

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ANDERSCH and Gibson[1] have suggested certain modifications in the determination of the plasma proteins as a result of their interesting observation that heating the blood proteins for a short time in an alkaline solution intensifies the degree of color obtained with the Folin phenol reagent. The writers, on reinvestigating this subject, reached somewhat different conclusions, and did not obtain the same tyrosine conversion factors as were found by Andersch and Gibson. Therefore, it appears desirable to present briefly the results that were obtained and to describe certain modifications in the determination of total serum protein, albumin, globulin, and fibrin, which were suggested by this work.

That heating considerably increases the chromogenic values which are obtained with the serum proteins was readily confirmed, but a considerably shorter time of heating was found to be sufficient to obtain a maximum color than the half hour recommended by Andersch and Gibson. Representative results on the change in color value obtained on heating human and dog serums are shown in Fig. 1. As this figure shows, a constant color is reached within five minutes on heating the protein in a boiling water-bath. However, in developing the procedures of the revised method to allow for a margin of

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safety, it was decided to employ ten minutes for heating, a time which has proved to be amply satisfactory. The innovation of heating distinctly improves the colorimetric method, in that, more reproducible color values are obtained and samples of serum which are several days old can now be successfully used for analysis without the loss in color which had been noted in the older procedure.[4]

Andersch and Gibson propose to return to the use of $(NH_4)_2SO_4$ for the salting out of the proteins, rather than continue with Na_2SO_4 which requires a temperature of over 30° to keep it in solution in the required concentration. In our estimation, this change is not desirable because more time is required, and it is more difficult to filter off the precipitated globulin. With the salt

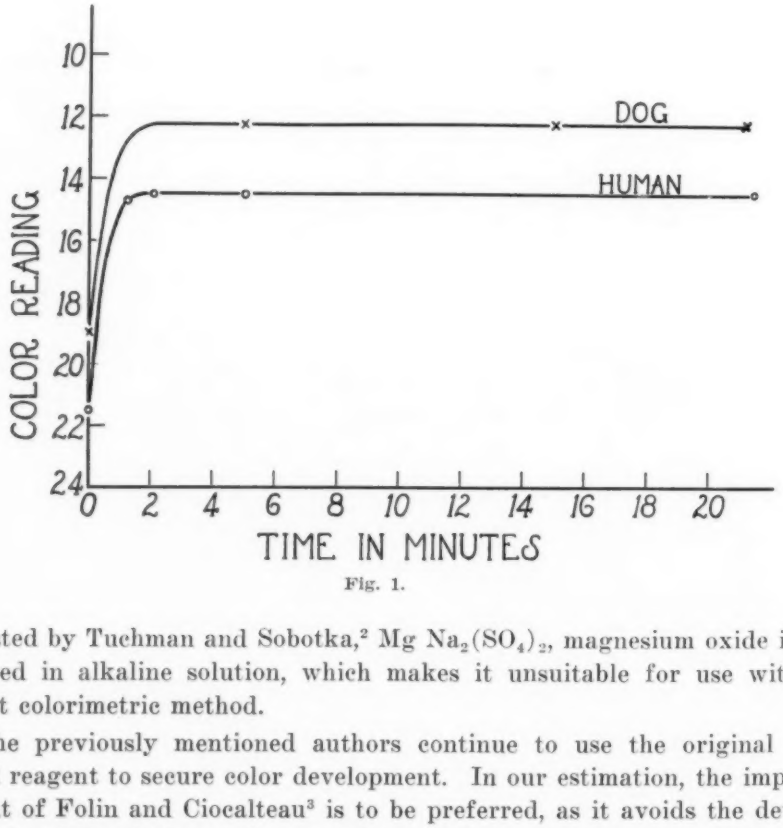

Fig. 1.

suggested by Tuchman and Sobotka,[2] Mg $Na_2(SO_4)_2$, magnesium oxide is precipitated in alkaline solution, which makes it unsuitable for use with the present colorimetric method.

The previously mentioned authors continue to use the original Folin phenol reagent to secure color development. In our estimation, the improved reagent of Folin and Ciocalteau[3] is to be preferred, as it avoids the development of turbid solutions. A comparison of the tyrosine factors given in this paper with those of Andersch and Gibson indicates also that even with the new heating procedure, a somewhat greater color development is obtained with the Folin-Ciocalteau reagent.

EXPERIMENTAL

Except for the heating in alkaline solution, the procedures and the reagents used are, in general, the same as those described by Greenberg,[4] and are published in the laboratory textbooks of Hawk and Bergeim,[5] Peters and Van

Slyke.[6] A convenient bath in which to carry out the heating is formed by a large beaker covered with a metal plate containing holes of sufficient size to hold the test tubes suspended in the water without allowing them to slip through. The water should be kept boiling briskly during the heating period.

Total Protein.—The serum is diluted in the proportion of 1 to 10 with 0.9 per cent NaCl. Pipette 2 c.c. of the diluted serum into a 20 c.c. test tube, and add about 5 c.c. of H_2O and 2 c.c. of 5 N NaOH. Mix the contents and heat in the boiling water-bath for ten minutes. Now insert a funnel into a 50 c.c. graduated flask and transfer the contents of the tube into the flask, washing out the tube with several portions of distilled water. Add 3 c.c. of the phenol reagent, made up to the graduation mark, and carry out the color comparison against a similar standard containing 5 c.c. of the standard tyrosine solution.

Albumin and Globulin.—The globulin from 0.5 c.c. of protein is salted out in the manner described by Greenberg. Five cubic centimeters of the filtrate containing the albumin are pipetted into a 20 c.c. test tube, then 5 c.c. of water and 2 c.c. of 5 N NaOH are added. After mixing the contents, the tube is heated in the boiling water for ten minutes. Dilution with water is desirable because otherwise the high concentration of sodium salt present causes a precipitation of some of the albumin. The rest of the procedure is the same as is given above for the total protein.

To estimate the globulin, after washing the precipitated globulin on the filter paper with Na_2SO_4 solution, insert the funnel into a test tube of 25 to 30 c.c. capacity. Puncture a small hole in the bottom of the filter paper and wash down all the globulin with approximately 0.01 N NaOH. Then unfold the paper and wash off any adhering protein into the test tube. The total volume resulting from this should not be more than 15 or 20 c.c. Now add 2 c.c. of 5 N NaOH and heat the tube on the boiling water-bath for ten minutes. Next, allow the contents of the tube to cool, decant into a 50 c.c. volumetric flask, and wash out with a few cubic centimeters of water. Add 3 c.c. of phenol reagent and compare as usual.

Fibrin.—The plasma from oxalated blood is used and the fibrin is separated after the manner of Cullen and Van Slyke.[7] Pipette 1 c.c. of plasma into a 50 c.c. cylinder, and add 30 or 40 c.c. of 0.9 per cent NaCl and 1 c.c. of 2.5 per cent $CaCl_2$ solution (prepared from the anhydrous salt). Mix the contents and allow the solution to stand for twenty to thirty minutes. The clot is picked up by gently rotating a glass rod through the clotted solution. Remove the clot to a dry piece of filter paper and press out the adhering liquor as completely as possible. Place the dry protein into a conical 15 c.c. centrifuge tube, and add 10 c.c. of water and 1 c.c. of 5 N NaOH. Now mix and heat the tube on the boiling water-bath for ten minutes. The fibrin will be dissolved, leaving behind a suspension of calcium oxalate. This is centrifuged down and the supernatant liquid is transferred to a 25 c.c. volumetric flask. Wash out the tube with water and transfer the wash water to the volumetric flask through a small filter to prevent the transfer of the calcium oxalate. Now add 1.5 c.c. of phenol reagent and compare against a standard in a 50 c.c. volumetric flask containing 3 c.c. of the tyrosine solution.

Conversion Factors.—The factors to convert the colorimetric readings to their respective protein values and their standard deviations are given in Table I. The figures are based on the analyses of the blood of eight normal young human adults and five dogs. The factors were determined by running parallel nitrogen determinations on the blood proteins by the micro-Kjeldahl method of Pregl. In these determinations total protein and albumin were determined by precipitating the protein fractions with 10 per cent trichloracetic acid previous to digestion and washing out the nonprotein nitrogen. Globulin was estimated by difference.

TABLE I

TYROSINE EQUIVALENTS FOR CONVERTING COLORIMETRIC READINGS TO GRAMS OF RESPECTIVE PERCENTAGE OF PROTEIN*

		TOTAL PROTEIN	ALBUMIN	GLOBULIN	FIBRIN
Human	F	11.5 ± 0.2	11.8 ± 0.2	10.5 ± 0.25	11.55 ± 0.2
	A	5.75	4.72	2.10	0.347
Dog	F	11.35 ± 0.25	11.6 ± 0.4	10.05 ± 0.4	11.1 ± 0.45
	A	5.68	4.64	2.01	0.333

*Doubt has been thrown on the validity of the tyrosine method of estimating the serum proteins in the blood of patients suffering from nephritis and nephrosis by Tuchman and Sobotka because it is claimed there is an alteration in the values of the tyrosine equivalents. We have not had the opportunity to test this point with our revised procedures. However, Andersch and Gibson report satisfactory results on such patients when the proteins are heated in alkaline solution.

The factors under the heading F are for use in the equation,

$$\frac{S}{U} \times T \times \frac{100}{V} \times \frac{F}{1{,}000} = \text{protein (in per cent)},$$

in which S is the setting of the standard, U is the reading of the unknown, T is the mg. of tyrosine in the standard solution, V is the aliquot volume of serum or of plasma used, and F is the factor for the particular protein fraction which is being analyzed. If the values of T and V are maintained at the levels described in the procedures given above, namely, T at the value of 1 mg. of tyrosine for the analysis of total protein, albumin, and globulin, and 0.6 mg. for fibrin, and the sample values of V are kept at 0.2 c.c. for total protein, 0.25 c.c. for albumin, 0.5 c.c. for globulin, and 1 c.c. for fibrin, then the equation may be reduced to the form

$$\frac{S}{U} \times A = \text{protein (in per cent)}.$$

The values of A for the respective protein fractions are given under this heading in Table I.

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A SIMPLE, EFFICIENT, AND INEXPENSIVE DEVICE FOR DRYING PIPETTES AND OTHER LABORATORY GLASSWARE*

HENRY FOY, SALONIKA, GREECE

A SIMPLE and easily manipulated pipette drier can be made from the ordinary electric hair drier, as used by ladies' hair dressers for drying hair. This instrument produces a rapid stream of hot or cold air which it blows out

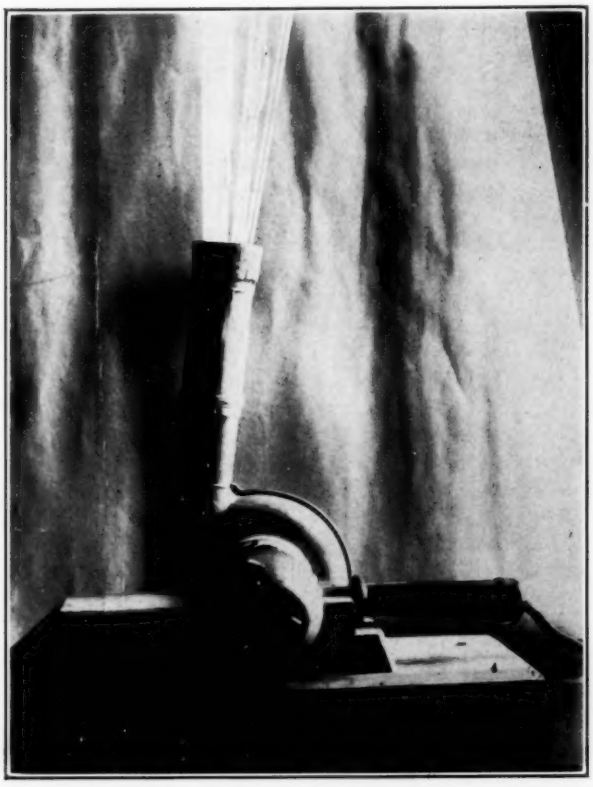

Fig. 1.

with considerable force, and pipettes can be dried by means of this instrument in a few seconds, six or eight at a time. All that is necessary is to fit a tin

*From the League of Nations Malaria Research Laboratory.
Received for publication, April 8, 1935.

nozzle over the spout of the blower, and to fit a cork into this nozzle in which six or eight holes are bored to take pipettes of various sizes. We have used an ordinary piece of pliable zinc, about 4 or 6 inches long, turned into a tube by rolling, and bound round the exit of the blower with copper wire; no doubt a more elegant arrangement could be devised, but this is quite satisfactory. We have placed our blower on a wooden base, with the blowing end uppermost, as shown in Fig. 1; if such a devise is not used the machine is awkward to hold still on account of its shape.

It is as well not to let the hot air run for more than a few seconds, as the nozzle of the blower being closed, the machine is likely to get very hot, and ruin the working parts, besides the danger of breaking the pipettes. A few seconds of hot air followed by a few of cold are sufficient to dry acetoned pipettes. By means of this device hundreds of pippettes can be dried in a very short time. We have used one of these in this laboratory for the past three years without any trouble whatever.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MENINGITIS, Meningococcic and Non-Meningococcic in New-Born and in Young Infants, Ravid, J. M. Am. J. Dis. Child. **49**: 1282, 1935.

Meningitis in the newborn and in young infants is characterized by being atypical, protein, and masked in its clinical manifestations.

The diagnosis is often missed and the condition mistaken for acute gastroenteritis, congenital debility, marasmus, tetany, etc.

Illustrative cases are reported in which signs of involvement of the central nervous system were scant or absent and in which the clinical picture centered around the gastrointestinal or respiratory systems.

A review of the literature on this subject was made which emphasized the relative rarity of meningitis at this age. A statistical study of all cases of meningitis in children during a four-year period at the New York Post-Graduate Medical School and Hospital showed that only 2.5 per cent of the cases of the meningococcic and 4.1 per cent of those of the nonmeningococcic variety occurred in infants three months of age and younger.

The clinical picture as it differs from that in older children and in adults is given in detail. In the absence of pathognomonic signs and when meningeal signs are not found, the following nonspecific signs and symptoms may be suggestive of meningitis: an irregular fever in the presence of gastrointestinal disturbances of any sort which occur without any demonstrable cause and which do not respond readily to treatment; in addition, hyperesthesia, irritability, somnolence, tenseness or bulging of the fontanel, distention of the veins of the neck, incessant crying and a dissociation between the pulse and respiratory rates and the temperature.

The pathogenesis of meningococcic and nonmeningococcic meningitis is discussed. The various portals and modes of entry, and sources and means of spread of infection are cited. The different causative organisms of pyogenic meningitis and their relative frequency are reviewed.

The prevalence of meningitis due to *B. coli* and related organisms in the newborn and its rarity in older children is emphasized, and explanatory factors are cited.

The anatomicopathologic features are practically the same in the newborn as in older children.

The prognosis of all forms of meningitis, especially of the pyogenic varieties at this age, is extremely poor. Mortality figures for each variety are given, the lowest being for meningococcic mengitis, which is 48 per cent or higher. The high mortality from meningococcic meningitis is due mainly to the difficulty of diagnosis and the delay in the institution of treatment. Cases of recovery from nonmeningococcic meningitis that was spontaneous or that followed specific or nonspecific treatment are cited from the literature.

A diagnosis in the absence of any pathognomonic signs is extremely difficult. Some of the signs and symptoms that may be helpful are cited. The most reliable diagnostic method is still spinal puncture, and it should be resorted to frequently.

The treatment of meningococcic meningitis in the newborn and in young infants is the same as in older children, the conservative line of treatment being the safest and best one to follow. The ventricular and cisternal routes should not be used indiscriminately and should be resorted to only in event of blockage of the circulation of the cerebrospinal fluid. Treatment of nonmeningococcic meningitis is less encouraging, but there are nevertheless some indications that new methods of treatment forecast a more favorable progress in this variety also.

HYPERGLYCEMIA, Evaluation of, in the Treatment of Diabetes, Mosenthal, H. O. J. A. M. A. 105: 484, 1935.

High blood sugar in the absence of glycosuria is not due to diminished excretory activity of the kidney, nor is it significant of nephritis, but it is a harmless anomaly resulting from an unusually active reabsorption of sugar from the urine in the renal tubules.

While a normal blood sugar level and freedom from glycosuria are among the ideal objectives of therapy in diabetes, there are certain circumstances in which hyperglycemia and glycosuria should not be completely corrected:

a. A normal blood sugar level and freedom from glycosuria should not be gained by underfeeding, since malnutrition favors the development of arteriosclerosis and is one of the contributory factors in producing diabetic coma.

b. Small amounts of sugar may be eliminated in the urine without detrimental results, as shown in renal glycosuria. It is important to recognize this because, in cases of diabetes with a comparatively low renal threshold, there is with rigorous insulin control the possibility of repeated hypoglycemic reactions, which in all probability are injurious in both their immediate and their remote effects and, consequently, should be avoided even at the sacrifice of intermittent glycosuria.

c. Hyperglycemia without glycosuria, according to the available evidence, not only has no damaging effect on the heart and other tissues but is a necessary stimulus for the proper assimilation and oxidation of dextrose in many persons, both diabetic and nondiabetic.

A prolonged, marked glycosuria with its attendant polyuria and dehydration is responsible for the diminished resistance to infectious processes, arteriosclerosis, formation of cataract, malnutrition, acidosis and coma, which are so characteristic of diabetes. Hyperglycemia without glycosuria is not a cause for these complications in diabetes.

PYURIA, Significance of, in Children, Hepler, A. B., and Scott, R. T. J. A. M. A. 105: 499, 1935.

There is a tendency among pediatricians to evaluate the importance of pyuria in children on a quantitative basis and to assume that a few pus cells are normal or indicate contamination and that a large number point to urinary tract infection or disease.

Various arbitrary limits of normal are given without qualification as to the method of collection or examination. No systemic study of a large number of children has been reported that would definitely confirm or refute many of the assumptions that appear in the literature on this subject.

To this end 694 infants and children were examined during a period of nine months in the Children's Orthopedic Hospital, from which study we conclude that:

Catheterization is imperative in urinary diagnosis in children. Ninety-nine per cent of 692 children had pus in the voided urine and only 13 per cent in the catheterized.

The amount of pus in a urine properly collected is no indication either of the kind or of the severity of urinary tract disease. In the twenty-four children in this group with demonstrable urinary tract disease, exactly similar lesions existed with pus counts that varied from less than 1 per high dry field to more than 20 per high dry field. Fifty per cent of the children with demonstrable urinary tract disease and a number with advanced lesions and severe infections had pus in amounts well under what is frequently set forth as a normal count.

If a persistent or recurrent pyuria, no matter what the cell count, is taken as a criterion for a complete renal study, a number of children will be subjected to what is apparently an unnecessary examination, for only 37 per cent of the 64 with pus in the catheterized specimen had demonstrable urinary tract disease. However, as can be noted in Table III, the amount of pus is no guide as to the necessity for cystoscopy. Many of the obstructive lesions are "silent" and a few pus cells the only indication of a pathologic process, so that it is undoubtedly a "greater error to miss making an early diagnosis through neglect of proper examination than to mistake the indication and subject the child to what is apparently an unnecessary examination."

Many urologic lesions in children are "silent" or have misleading symptoms. Only 9 of the 26 children in the study who were found to have urinary tract disease had subjective

symptoms that were in any way referable to the urinary tract. In only 10 was there any presumption of a urinary lesion. The remaining sixteen were discovered through the routine study of pyuria.

Urinary complications in children with bone and joint tuberculosis are not common, and prolonged immobilization on frames or in casts does not tend to urinary stasis, infection and stone formation. Forty-one of the children examined had bone or joint tuberculosis. In none were tubercle bacilli found in the urine after repeated and careful examination. Only 7 had pus in the urine, and none of these had any demonstrable urinary tract disease.

In determining the type of organisms in 183 urines, the fresh smear and cultures agreed in 170, or 92 per cent.

MONONUCLEOSIS, Infectious, Davidsohn, I. Am. J. Dis. Child. **49:** 1222, 1935.

Davidsohn describes a rapid method for the heterophile antibody test which may be read in four hours:

The serum dilutions range from 1:2.5 to 1:5,120, or higher if necessary. The quantity of the dilution used is 0.25 c.c. One-tenth cubic centimeter of a 2 per cent suspension of sheep cells is added. Narrow test tubes, measuring 75 mm. in length and 12 mm. in the external diameter, are used in order to make possible the reading of the results with a microscope. The test tubes are shaken and placed in a water-bath at 38° C. for one hour and then in an ice box for one hour. At the end of two hours, the test tubes are shaken until the sediment is suspended, and the results interpreted as +++ and ++ are estimated with the naked eye. The result read as + can be fairly accurately estimated with the naked eye, but a finer, more accurate reading is obtained by placing the test tube flat on its side on the stage of the microscope and looking at the cell suspension with the low power of the objective. The titers obtained with the microscope are from one to two dilutions higher than those read with the naked eye.

LYMPHOGRANULOMA INGUINALE, D'Annoy, R., Von Haam, E., and Lichtenstein, L. Am. J. Path. **21:** 737, 1935.

Seven endemic strains of the virus of lymphogranuloma inguinale have been isolated and transmitted to animals.

Intracerebral inoculation of infectious material produced a typical meningoencephalitis in the common marmoset while the rhesus monkey proved resistant to such inoculations.

The virus could readily be transmitted to white mice, biweekly inoculations allowing upkeep of its maximal virulence.

Brain emulsions of infected monkeys and mice act as excellent stable and sensitive antigens for the specific diagnostic intradermal reaction of Frei.

Twenty-eight per cent of infected guinea pigs showed enlargement of the regional lymph glands with histologic lesions consistent with the disease.

Experiments with sheep, chickens, and frogs indicate that the virus can infect sheep; that its virulence can be preserved in the brains of chickens; and that frogs cannot be infected.

Cultivation of the virus after the method of Tamura was possibly confirmed.

The virus of lymphogranuloma inguinale, as encountered endemically in the poorer negro population of Louisiana, shows rather identical behavior concerning animal transmission as the virus strains studied in other parts of the United States and abroad.

HYPOGLYCEMIA in the New-Born, Higgons, R. A. Am. J. Dis. Child. **50:** 162, 1935.

Two cases have been reported which appear identical in every respect except in the family history. It is an open question whether the insulin taken by the mother of the patient in the first case had any effect in producing the hypoglycemia in her baby. Certainly there was no exogenous insulin in the second case.

It seems likely that some of the cases of convulsive attacks during the first few days of life which are now considered to result from birth injury may be due to hypoglycemia. The author feels that a reading of the blood sugar should be made in all these cases before

a diagnosis is made. It seems reasonable to suppose that without proper treatment one or both patients might have died owing to a continued fall of the blood sugar level. Babies take food with great difficulty after severe hypoglycemia develops, and without the use of gavage and parenteral administration of sugar the deficiency of sugar in the blood might not be corrected.

NEPHRITIS, Hemorrhagic, Infection and, Winkenwerder, W. L., McLeod, N., and Baker, M. Arch. Int. Med. 56: 297, 1935.

An analysis of the relationship between infection with *Str. hemolyticus* and hemorrhagic nephritis in a series of 78 cases observed for from one to eight years is reported. Twenty-two of the patients are well, 21 are in the latent stage of the disease, 17 are in the progressive stage, and 19 are dead.

Infections, usually of the upper respiratory tract, caused by *Str. hemolyticus* (alpha type, in a few cases), preceded the onset of hemorrhagic nephritis in the great majority of cases in this series.

Pneumococcic pneumonia, rheumatic fever and syphilis were not considered as causes of hemorrhagic nephritis in this series.

Str. hemolyticus is apparently related to the progress of nephritis; for the numbers in which it occurred diminished markedly during recovery but persisted during the progressive stages of the disease. In 11 cases, however, hemolytic streptococci were not shown at any time during the course of the disease.

The cases of hemorrhagic nephritis which were preceded by acute infection manifested by both local and constitutional reactions almost always ended in recovery or entered the latent phase; the cases of nephritis associated with chronic infection at the onset almost always became progressive.

The prodromal period between the beginning of infection and the onset of nephritis varied from three to twenty-eight days; the most frequent interval was seven and the average was ten and nine-tenths days.

A seasonal variation in the onset of hemorrhagic nephritis coincided with months during which respiratory infections are most frequent in Baltimore.

Exacerbations of nephritis in most instances followed streptococcic infections of the upper respiratory tract; these exacerbations occurred more frequently in the latent and in the progressive stages of the disease. The prodromal period between infection and the exacerbation of the nephritis was usually from twenty-four to forty-eight hours, in contrast to the prodromal period at the onset of nephritis. Exacerbations also occurred without relation to infection, and also after operative procedures.

Streptococcic infections which occurred during the convalescent stage did not prevent recovery from nephritis; and after recovery was established they rarely caused relapses; whereas the "carrier state," with or without recurring evidences of active infection, was characteristic of the progressive stage of hemorrhagic nephritis.

Surgical removal of foci of infection may fail to influence the outcome of hemorrhagic nephritis.

It is suggested that recovery from or progression in hemorrhagic nephritis is related more to the character of the causal infection: a severe acute infection presages a favorable course; a chronic infection, an unfavorable outcome. It is also suggested that patients with acute infection possess the capacity to react to *Str. hemolyticus*, so that some form of resistance to the infection develops, and as a result recovery from nephritis follows and disappearance of the organism occurs. In patients with chronic infection, this capacity to react to streptococcic infection is lacking, a resistant state does not develop, and as a result the organism persists and the nephritis becomes chronic.

MERCUROCHROME, Action of, on Normal Human Skin and in Infected Wounds, Hill, J. H. J. A. M. A. 105: 100, 1935.

There is urgent need for standard methods of studying the in vivo action of antiseptics for special uses. In this paper methods are suggested which, on further refinement, might serve as bases for such standards.

In regard to the action of antiseptics applied to the skin, it is shown that:

a. Under conditions of practical use no antiseptic studied can invariably sterilize heavily infected skin.

b. Aqueous solutions of antiseptics are not as a rule suitable for preoperative skin sterilization.

c. Both the 2 per cent tincture and 2 per cent aqueous mercurochrome solutions are bactericidal and bacteriostatic on human skin. The 2 per cent tincture of mercurochrome is superior to the aqueous solution on the skin, as has been shown previously. Only the tincture has been advocated for preoperative skin sterilization.

d. If comparisons are to be made between the bacteriostatic actions of preparations of iodine and mercurochrome on the skin, the order of efficacy, according to the results of our experiments, is as follows: first, the 2 per cent tincture of mercurochrome; second, the 7 per cent tincture of iodine, not removed with alcohol; third, the 2 per cent aqueous solution of mercurochrome; fourth, the 7 per cent tincture of iodine, removed with alcohol.

In regard to the use of antiseptics in wounds, it is shown that:

a. It is improbable that a single application of any known antiseptic will sterilize a heavily infected wound.

b. There is evidence that while both the tincture and aqueous solutions of mercurochrome are bacteriostatic in heavily infected wounds, the aqueous solution, under the conditions of the tests, is superior to the tincture of mercurochrome and to the other antiseptics tested, in that it keeps the bacterial count lower and does not interfere with phagocytosis.

DIPHTHERIA, Rapid Method for Identification of B. Diphtheriae, Brahdy, M. B., Lenarsky, M., Smith, L. W., and Gaffney, C. A. J. A. M. A. 104: 1881, 1935.

Sterile cotton swabs are impregnated with undiluted unheated horse serum to which no preservative has been added. The swabs are then squeezed lightly against the sides of the tube to remove any surplus serum. They are removed and lightly heated over a flame to obtain surface coagulation and, possibly, to destroy any antibodies in the serum. The swabs are then used to take the nose and throat cultures in the routine manner. Instead of being implanted on a culture medium the swabs are put in dry sterile tubes, placed in the incubator and examined at the end of two and four hours. The physician's vest pocket may serve as an incubator. At the end of the incubation period, smear preparations are made on slides directly from the swab.

MENINGITIS, Acute Aseptic, Viets, H. R., and Watts, J. W. J. Nerv. & Mental Dis. 80: 253, 1934.

The disease known as acute aseptic meningitis, first clearly described in 1925 by Wallgren, has now found a well-marked place in medical literature.

Patients with this disease, described by the authors in 1929, have been reexamined and found to conform with Wallgren's description.

New cases have been added to the authors' series, making a total of fourteen.

Contrary to the authors' opinion expressed in 1929, they now believe that the changes in the cerebrospinal fluid are not exclusively lymphocytic, although essentially so. Clots, also, may be found in the fluid.

The most important point in differential diagnosis between this disease and tuberculous meningitis is the constantly normal sugar and chloride content of the cerebrospinal fluid in acute aseptic meningitis, contrary to the findings in tuberculosis.

The disease should be considered as a clinical entity, as no definite relationship has been shown to exist between it and any other diseases.

The essential characteristics of the syndrome are: acute onset; nonpurulent (lymphocytic) character; inflammation of meninges.

The disease, however, has other characteristics: it is benign, self-limited, seldom, or perhaps never, results in late manifestations. It is mildly epidemic, and the cerebrospinal formula is strongly lymphocytic without decrease in the sugar or chloride content.

Acute aseptic meningitis *begins acutely or subacutely.*

The course of the disease varies widely in both severity and duration, although all patients recover within a period of a few weeks. The more marked symptoms, such as coma and convulsions, usually disappear after the first or second withdrawal of cerebrospinal fluid.

Examination of the cerebrospinal fluid is essential to diagnosis. In some instances where the disease is not severe, the changes in the fluid are mild. There is a slight pleocytosis, essentially lymphocytic in formula, without polymorphonuclear leucocytes. The pressure is normal; the protein is slightly increased, but not markedly so; and the sugar and chloride contents remain within the normal limits. Most of the cells in the fluid are small lymphocytes, although occasionally one finds a few large lymphocytes.

In general, the cerebrospinal fluid varies directly with the severity of the illness. In the milder cases the cells are few in number and exclusively *lymphocytic;* in the more severe cases there is a great increase in cells, up to 600 or 700 per c.mm., and polymorphonuclear leucocytes are frequently found. The presence of these latter cells indicates a severe illness, their reduction in number and final disappearance are coincident with recovery, and a careful recording of them, therefore, is helpful to the prognosis. The most important finding, however, in the cerebrospinal fluid is the constant sugar and chloride content. This is in marked contradistinction to the findings in tuberculous meningitis and is the chief laboratory test which is helpful in the differential diagnosis of the two diseases.

Although the chloride content may be slightly decreased, it never varies much from the normal. The colloidal gold reaction falls in the meningitic zone. The Wassermann reaction is always negative; bacteria are not demonstrated by either smear or culture, and guinea pig inoculation gives negative results.

ASCHOFF BODY, Studies On the Myocardial. II Life Cycle, Sites of Predilection, and Relation to Course of Rheumatic Fever, Gross, L., and Ehrlich, J. C. Am. J. Path. 10: 489, 1934.

There has been presented in this report a study of the life cycle of the myocardial Aschoff body, based on an examination of the clinical records and autopsy material from 70 cases that presented Aschoff bodies in the myocardium. It appears that these specific lesions pass through three stages in development. The earliest phases, represented by small cell coronal and reticular Aschoff bodies, have been found to occur up to the fourth week after the onset of the illness. The middle phases, represented by large cell coronal, syncytial coronal, mosaic and large irregular cell polarized Aschoff bodies, have been found to occur between the fourth and thirteenth week after the onset of the illness. The late phases are represented by polarized Aschoff bodies which occur from the ninth to the sixteenth week after the onset of the illness, and subsequently by fibrillar Aschoff bodies which occur after the thirteenth week of the illness.

The earliest types of specific lesions are apparently influenced in their response by the reactivity of the tissue, depending on whether there has or has not been a previous attack of rheumatic fever, and also by the state of the collagen present in the interstices between the myocardial bundles. As a consequence, the evolution of the lesion may follow one of two main courses, determined by the initial lesion. The latter may occur in the form of the reticular or the small cell coronal Aschoff body. The final phases of the life cycle of the Aschoff body are common to both main courses.

Dividing the material into four groups representing different clinical courses, there appears to be some change both in the incidence of the types of Aschoff bodies present in the myocardium and in their localization. The findings reported here, however, can by no means be considered as furnishing sufficient statistical evidence on which to base final conclusions on this point. That the tempo of the life cycle may be considerably faster or slower than what has been described in this report seems very probable. Some of the stages in the "model" of the life cycle presented by the authors may be absent in some cases, abbreviated in others, or indeed, appear in the reverse order from what we have suggested. These facts can be determined with greater accuracy only after examining a much more extensive series of cases and, in the last analysis, must await confirmation by the hitherto unsuccessful transmission of this disease to animals. It is hoped, however, that further studies will be made along these lines in order that some of these interesting relations may be placed on a firmer footing.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Food and Beverage Analyses*

ALTHOUGH small and compact, this volume contains a remarkably comprehensive survey of foods, including those canned and packaged, somewhat of an innovation in texts of this character.

Not only the nutritive and caloric values of foods and beverages are tabulated but also their mineral, iodine and vitamin content. The alcoholic table is as of 1935.

In each instance, not only the percentage proportion of the various elements, but also their content in grams per each portion of a specific food is given.

This volume should be of great value to all those interested in nutrition and home economics and, of course, especially to those interested in the preparation of special diets for specific purposes.

It may be regarded as an authoritative text, perhaps the most comprehensive yet available.

Clinical Parasitology and Tropical Medicine†

THE purpose of this volume is to present a survey of the subjects, which, while not all-inclusive, will nevertheless discuss in an adequate manner the present status of this specialized field of medicine.

The major divisions of the text consider: Part I. General Considerations of Etiology and Pathology, environmental conditions, laboratory diagnosis, and treatment. Part II discusses the diseases due to protozoa; dysenteries, flagellate diarrhea; malaria, trypanosomiasis, leishmaniosis, and spirochetiosis and trepanemiasis. Part III is concerned with the diseases caused by metozoa: nematodes, trematodes, and cestodes; Part IV discusses the bacterial diseases most common to the tropics; Part V. Diseases of Undetermined Etiology under which are included filtrable viruses, metabolic disturbances, and vitamin deficiencies; while Part VI concerns a discussion of climatic diseases and animal poisons, including heat stroke and poisonous bites and stings.

It will be seen that the authors have set themselves a difficult task. There is much in the book that is useful, especially, perhaps, as concerns laboratory diagnosis. The specialist in this field, however, will find the text somewhat less than his requirements demand, while the student will not be in a position either to note or estimate the importance of that which is omitted or touched upon somewhat briefly.

The sections on treatment describe the authors' methods at some length, others receiving but brief mention in many instances, and in some cases being omitted entirely.

There are some typographical errors, principally in the spelling of names, etc., which require correction; these, together with the factors already noted, will be corrected in future revisions.

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*Food and Beverage Analyses. By Milton A. Bridges, M.D., Director of Medicine, Department of Correction Hospitals, New York, Assistant Professor of Medicine and Lecturer in Therapeutics, New York Post-Graduate Medical School, etc. Cloth, 248 pages, Lea and Febiger, Philadelphia, Pa.

†Clinical Parasitology and Tropical Medicine. By Damaso de Rivas, M.D., Professor of Parasitology, Graduate School of Medicine, University of Pennsylvania, etc., and Carlos T. de Rivas, M.D., Pathologist, Santa Toma Hospital, Panama. Cloth, 367 pages, Figs. 144, 1 colored plate. Lea and Febiger, Philadelphia, Pa.

The Design of Experiments*

THIS is a companion volume to the author's previous work "Statistical Methods for Research Workers" and to the general body of scientific workers will be found of equal interest and value.

Demonstrations of Physical Signs in Clinical Surgery†

HERE, without doubt, is an excellent book which should be in the hands of every physician.

That an accurate diagnosis is the essential prerequisite to intelligent treatment is obvious, just as it is equally obvious that careful and thorough examination is essential to the formation of a diagnosis. But it may sometimes be questioned if physical examinations are always as careful and thorough as they might be or if all physicians bring to them the many "tricks of the trade," so to speak, which have evolved from experience.

Despite the fact that the title might suggest that the book is primarily of value to the surgeon, it is, on the contrary, an extremely valuable book for the physician in general. For, in many instances, the conditions ultimately referred to the care of the surgeon are first seen and must first be recognized by the physician.

The book is obviously built upon a varied and extensive experience well and ably presented to the reader.

The style is clear, there is no ambiguity, and the author speaks clearly and simply. The illustrations are not only numerous and well reproduced but they illustrate clearly.

This volume can be recommended without reserve as one well fitted for the physician at large to use as a desk companion, frequent reference to which will greatly benefit both doctor and patient.

It is among the best of all the books of its kind which this reviewer has encountered.

Tumors of the Urinary Bladder‡

IN THIS excellent monograph Dr. Beer reviews the subject of tumors of the urinary bladder based upon an experience covering 650 cases encountered during the last 25 years, 388 being definitely malignant tumors and 262 definitely benign. In this series males were more frequently attacked than females, the ratio being 4.5 to 1. The present consensus of urologists is in favor of cystoscopic high frequency electrocoagulation in the treatment of benign bladder tumors, while surgery is still the choice of the majority for malignant cases.

Beer believes that surgery, despite its higher mortality, is still the more promising method of attack and offers the better chance of permanent cure. He practices total excision and hopes that improvement in technic may eventually lead to decreased mortality.

This little volume is evidently the fruit of varied and extensive experience and may well be read with interest and profit by all who are interested in this subject.

Well printed and excellently illustrated it is a contribution of distinct value.

If it presents the experience of but one clinic, it is an experience which has been, not only extensive, but carefully recorded and analyzed.

There is a bibliography of 27 pages and also a satisfactory index.

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*The Design of Experiments. By R. A. Fisher, Golton Professor, University of London. Cloth, 252 pages, Oliver and Boyd, Edinburgh, Scotland and London.

†Demonstrations of Physical Signs in Clinical Surgery. By Hamilton Bailey, F.R.C.S., Surgeon, Royal Northern Hospital, London, etc. Cloth, ed. 5, 287 pages, 341 illustrations and colored plates. William Wood & Co., Baltimore, Md.

‡Tumors of the Urinary Bladder. By Edwin Beer, M.D., Visiting Surgeon, Mount Sinai Hospital, New York. Cloth, pp. 166, 52 illustrations, 8 in color. Wm. Wood & Co., Baltimore, Md.